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THE HARVEY LECTURES

DELIVERED UNDER THE AUSPICES OF

THE HARVEY SOCIETY OF NEW YORK

1913-1914

BY

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PROF. G. H. PARKER
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PREFACE

An apology is due our readers for the late appearance of this, the ninth volume of Harvey Lectures. For this delay in publication full responsibility must fall on the undersigned, who assumed the duties of editor on account of the absence in Europe of the Secretary, Dr. John A. Mandel.

We wish to express, as in former years, obligations for permission to reprint those lectures which have already appeared in medical and scientific journals: To the editors of Science, The Archives of Internal Medicine, and the American Journal of Medical Sciences for allowing us to republish, respectively, the lectures by Prof. Parker, Dr. Cole, and Prof. Schmidt.

April, 1915. Robert A. Lambert, *Acting Secretary.*

THE HARVEY SOCIETY

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE OF THE MEDICAL SCIENCES

CONSTITUTION

I.

This Society shall be named the Harvey Society.

II.

The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology, and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented.

III.

The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences residing in the City of New York. Associate members shall be such other persons as are in sympathy with the objects of the Society. Honorary members shall be those who have delivered lectures before the Society and who are neither active nor associate members. Associate and honorary members shall not be eligible to office, nor shall they be entitled to a vote.

Members shall be elected by ballot. They shall be nominated to the Executive Committee and the names of the nominees shall accompany the notice of the meeting at which the vote for their election will be taken.

IV.

The management of the Society shall be vested in an executive committee, to consist of a President, a Vice-President, a Secretary, a Treasurer, and three other members, these officers to be elected by ballot at each annual meeting of the Society to serve one year.

V.

The Annual meeting of the Society shall be held soon after the concluding lecture of the course given during the year, at a time and place to be determined by the Executive Committee. Special meetings may be held at such times and places as the Executive Committee may determine. At all the meetings *ten* members shall constitute a quorum.

VI.

Changes in the Constitution may be made at any meeting of the Society by a majority vote of those present after previous notification of the members in writing.

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ILLUSTRATIONS

A SHORT ACCOUNT OF THE ORIGIN AND SCOPE OF ELECTROCARDIOGRAPHY *

PROFESSOR AUGUSTUS D. WALLER
University of London

I HAVE chosen for my topic "the electrical action of the human heart." I shall of necessity give no more than a very brief sketch of a subject that has blossomed out considerably since the days—now nearly thirty years ago—when I first made acquaintance with it, and introduced it to the attention of physiologists.

It was in 1889 at the First International Physiological Congress at Basle that I first did so formally by a set demonstration, which I repeated the other day at Groningen at the Ninth International Physiological Congress and which I intended to repeat to-day here as an introduction to my argument. "Oh, just an old experiment," you say, "and we expected you to tell us something new." Well, I venture to claim that this old experiment, first shown at the First International Congress of 1889 and again at the last Congress in 1913, is still to all intents and purposes a new experiment, the proper understanding of which affords a key to the understanding of the whole story in its essential simplicity, freed from what I am afraid I must characterize as the intricately woven obscurities in which it is at present enveloped.

I cannot make that demonstration to-day. The apparatus by aid of which I was to have done so, which had been most liberally placed at my disposal for the purpose by the firm of Hans Thoma of Munich, has been completely smashed in transit —not merely broken in an ordinary fashion, but literally smashed beyond repair, as if by a coal-hammer. I hasten to say that this instrument smashing was not performed in the New York Custom House; it happened over in Europe—in Munich

* Delivered October 4, 1913.

or in London. I am very sorry; I am particularly sorry that the instrument-makers have suffered loss; I feel that we, men of science, are nowadays very helpless without the assistance of the skilled instrument-maker. I am very grateful indeed for the liberal intention of Messrs. Thoma, and not a little disgusted by the destruction of their beautiful instruments. It would have been a great pleasure to me to demonstrate my facts to-night and to enable you to judge of the excellence and convenience of these instruments. This is impossible. But happily some of my friends here share my regrets, and have promised to do their best to remedy the accident. You, Mr. President, have already come to my assistance, thanks to the magnificent instrument set up in your laboratory by Dr. Williams.

I will not spend time now describing instruments. A good many have passed through my hands in the last twenty-five years, from Lippmann's capillary electrometer to Einthoven's string galvanometer, of which the most perfect example I have ever seen is that set up by Dr. Williams in the physiological department of Columbia University. Every instrument has its advantages and disadvantages. One great advantage of the Columbia string is that it is splendidly steady and gives beautifully clear photographs; its only disadvantage is that it is a fixture and cannot therefore be brought here. On the other hand, the great advantage of the latest forms of electrocardiographic apparatus—viz., the Bock-Thoma oscillograph, which I intended to use to-night, and the Siemens-Halske, which I used the other day at Groningen—is that they are portable. Either of these instruments can be carried about in an automobile and set up in a few minutes in connection with a house current of 110 or 220 volts. The galvanometer proper is set up in connection with a condenser of, say, 30 to 50 microfarads which gets rid of the troublesome necessity of fiddling about with shunt and compensator. The instrument is just a penny-in-the-slot machine that can be used by a nurse or a lift-boy. All the physician has to do is to read the electrocardiograms when their photographs are finished.

And, by the way, I ask you to take it from me that for all practical purposes the photographs got with a condenser in circuit are quite as good as photographs got with a compensator in circuit. I satisfied myself of this fact some years ago when Professor Max Cremer recommended the use of a condenser. I shall also ask you to take for granted that in the values I shall quote due attention has been paid to possible differences of skin-resistance.

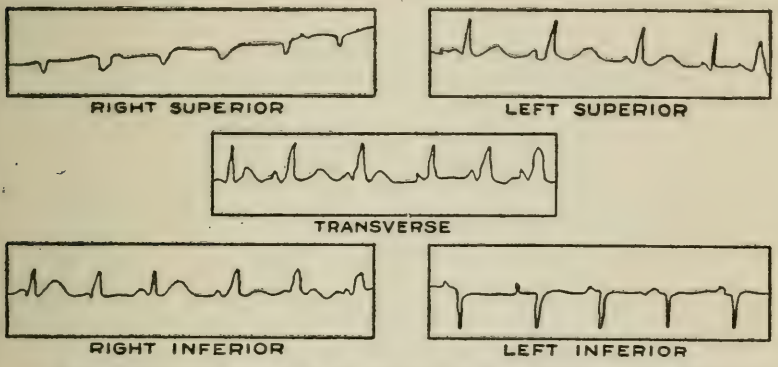

FIG. 1.—The five leads of A. D. W. taken by Dr. H. B. Williams by an Einthoven string galvanometer in the Department of Physiology of Columbia University at the College of Physicians and Surgeons, New York.

1 mm. ordinate = 0.0001 volt.

Systolic spike = −4.5 mm.	in the Right superior lead
10	Left superior
11	Transverse
4.5	Right inferior
−9	Left inferior

The calculation of the inferior axial angle is as follows:

$$\tan \alpha = 2\frac{4.5+9}{4.5-9} = \frac{6}{-1} = -6 \quad \therefore \alpha = 180° - 80° = 100°.$$

The Initial Experiment.—Every one knows to-day that with the two hands dipping in two basins of salt solution, which are connected to the two poles of an electrical indicator of sufficient sensitiveness and rapidity, rapid deflections of the indicator are visible at the rhythm of the pulse. Even if apparatus were now to hand I should hardly spend time in showing again this very familiar fact. But I should once more show the cardinal experiment of 1889. With one electrode in my mouth—say a silver spoon—connected to one pole of the galvanometer, I should dip first the left hand then the right hand into a basin of

salt solution connected with the other pole of the galvanometer through a second silver electrode. In the first case—mouth and left hand—*i.e.*, the left superior lead—you would see "large" electrical effects. In the second case—mouth and right hand—*i.e.*, right superior lead—you would see "small" electrical effects (or, maybe, none at all). The left superior is a favorable or strong lead, the right superior an unfavorable or weak lead. But as I cannot actually demonstrate this contrast to-day, I will show you in the lantern the records of my right and left superior leads taken this morning by Dr. Williams on the Columbia instrument (Fig. 1).

You recognize at once that the spikes are of smaller amplitude and of reversed direction on the right side as compared with the left. From their numerical values on the two sides we shall be able to estimate the angle which the superior current-axis of the heart forms with the vertical or mesial plane of the body. And in this respect it may be interesting to see what kind of value comes out here in New York, as compared with the values calculated for the same person by other kinds of instruments in London and in Groningen:

	R. Sup.	L. Sup.	Sup. α	Pulse Frequency.
August 29 in London with a Bock-Thoma	−5 −4	+12 + 9	67° 69°	84
September 1 in Groningen with a Siemens-Halske	−6	+14.5	67°	84
October 4 in New York with an Einthoven	−4.5	+ 9.5	70°	84

If you went on to try this simple experiment with a number of subjects you would find as the ordinary and regular event a large left-hand spike and a small right-hand spike ("spike" is my name for the tall brief peak of the electrocardiogram that occurs at the beginning of the ventricular systole; Einthoven designates it by the single letter R or by the three letters Q. R. S.).

Looking a little more closely into matters or, better still,

taking measurements of the photographic records of several subjects you would find considerable variations between the relations of these two magnitudes. With the mouth electrode connected to the galvanometer so that the left-hand spike reads "positive" you would find that in a good many cases the smaller right-hand spike was unreadably small or that it was actually less than zero, *i.e.*, reading in the opposite or "negative" direction. In my own case it is, as you see, negative. In that of Thomas Goswell it is practically zero. In many other cases it is positive but small.

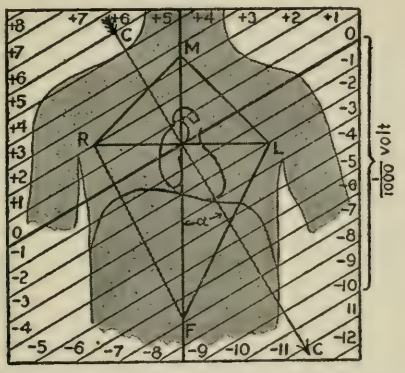

Fig. 2.—A typical heart with the electrical axis CC forming an angle of 30° with the vertical. The parallel lines at right angles to the current-axis CC represent equipotential lines parallel with the equator OO at regular intervals of 0.0001 volt.
ML and RF are the strong leads. MR and LF are the weak leads.

Now what is the meaning of this obvious difference between the spikes left and right? It is obviously due to the normal obliquity of the heart. The organ is tilted to the left. An imaginary arrow transfixing the heart from base to apex along its approximate anatomical axis forms more or less of an angle with the vertical. Our imaginary arrow in this axial direction represents a line of maximum current or current-axis (CC) resultant from the sum of the differences of potential that arise when the systolic wave of muscular contraction sweeps over and involves the entire ventricular mass beginning and ending at its basal side—near the auricles at the beginning and near the great arteries at the end, and for a briefer period involving

also the more or less extensive lump of muscle that we think about and sometimes loosely speak about as the apex of the ventricular mass. The order of contraction, as I tried to explain many years ago, is this: Base—Apex—Base. The base is twice negative at each systole of the ventricle, giving what I am accustomed to describe as the first and second ventricular waves of the electrocardiogram. The first ventricular is what I call the spike. Prefix to these two waves the well-known indication of the presystolic or auricular contraction (the wave designated

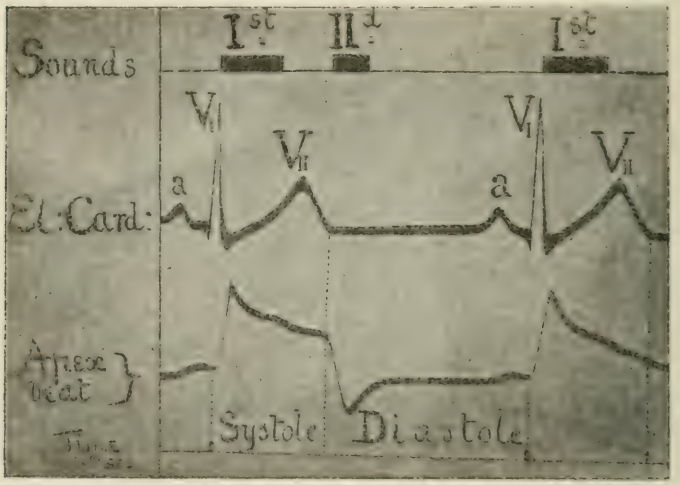

FIG. 3.—Diagram to exhibit the time-relations of the first and second ventricular waves of the electrocardiogram to the first and second sounds of the heart (upper line) and to the mechanical movement of the ventricular systole (lower line).

as P by Einthoven). You now recognize the typical electrocardiogram to the consideration of which I shall return in a moment when I have finished with the point we are just now considering, *i.e.*, the meaning of the relative magnitudes of the spikes in the records of the left and right superior leads.

These two spikes are to be regarded as expressing the maximal values of the potential difference occurring at the outset of ventricular systole on the two sides of the body, between mouth and left hand (the strong lead ML) and between mouth and right hand (the weak lead MR).

To any one accustomed to the more complicated nomenclature P.Q.R.S.T. first introduced in 1895 by Einthoven, and followed by most clinical writers, I am afraid that the simpler physiological nomenclature to which I adhere may seem a little strange. I only recognize three waves in the electrocardiogram —the auricular followed by two ventricular waves, first and second in correspondence with first and second sounds of the heart or the beginning and end of the ventricular systole. The accompanying diagram (Fig. 3) may help to remove any obscurity from this nomenclature.

It is obvious that the difference between strong and weak lead depends upon the obliquity of the heart's axis to the left. Obviously the right and left leads would be equal if the heart's axis were vertical. Equally obviously the difference between right and left leads will be greater as the axis forms a larger angle with the vertical. Simple physical considerations enable us to calculate what differences right and left will correspond with given angular inclinations of the cardiac axis, or *vice versa* to calculate the angular inclination of the electrical axis from given electromotive values of the right and left electrocardiograms. The problem is analogous to the mechanical problem of a bent lever to which unequal weights are suspended. In this case the tangent of the angular deviation of the lever calculated from the right and left weights will be proportional to the fraction $\frac{\text{Heavy}-\text{Light}}{\text{Heavy}+\text{Light}}$. In the case of the heart, the electrical angle calculated from the values of right and left spikes will be proportional to the fraction $\frac{\text{Strong}-\text{Weak}}{\text{Strong}+\text{Weak}}$. And without troubling you with the details of this very elementary problem, I shall make use of its results for calculating the electrical angle, superior and inferior, from the right and left values, superior and inferior, of the ventricular spike V_I. The letters R and L are here used to denote these right and left values.

For the superior angle the formula is: $\tan \alpha = \frac{L-R}{L+R}$.

For the inferior angle the formula is: $\tan \alpha = 2\frac{R-L}{R+L}$.

For these formulæ it has been assumed that the right and left spikes are perfectly synchronous. The vertical angle at M has been taken as 90°, that at F as 53°. If, following Einthoven, the angle at F is taken = 60° the formula for the inferior angle becomes $\tan \alpha = 1.73 \frac{R-L}{R+L}$.

Let us take some examples:

We have found, *e.g.*, for the left-hand spike with the lead ML a value of 0.8 millivolt, and for the right-hand spike with the lead MR a value = 0.2 millivolt, signifying a potential difference between M and L = 8 as compared with that between M and R = 2. Then: $\tan \alpha = \frac{8-2}{8+2} = 0.6, \quad \therefore \alpha = 31°$.

We have found, *e.g.*, that the value of the right-hand spike with the lead RF is double that of the left-hand spike with the lead LF.

Then: $\tan \alpha = 2 \frac{2-1}{2+1} = 0.67, \quad \therefore \alpha = 34°$.

Take lastly one of Einthoven's cases—that of Bak on page 297. He gives, *e.g.*, the values of the spike as 12.5 in Lead II, *i.e.*, with the right hand, and 9.3 for Lead III, *i.e.*, with the left hand. By means of his equilateral triangle and a geometrical projection of these magnitudes, he calculates the angle α as being 76° with the horizontal. Of course this is quite right but I must confess that I find Einthoven's calculation extremely troublesome. I much prefer to work the problem out from right and left values by my formula $\tan \alpha = 2 \frac{R-L}{R+L}$ or since Einthoven has taken the angle at F = 60°, by the slightly modified formula $\tan \alpha = 1.73 \frac{R-L}{R+L}$. Then:

$\tan \alpha = 1.73 \frac{12.5-9.3}{12.5+9.3} = 0.254$, therefore $\alpha = 14°$ with vert. or 76° with hor.

THE FIVE LEADS

The considerations dealt with up to this point may now be summarized and recapitulated as follows in accordance with the diagram of Fig. 2:

1. Transverse		R. hand – L. hand	RL
2. Right inferior	(Axial)	R. hand – L. foot	RF
	(Right lateral)	R. hand – R. foot	
3. Left inferior	(Equatorial)	L. hand – R. foot	LF
	(Left lateral)	L. hand – L. foot	
4. Right superior		Mouth – R. hand	MR
5. Left superior		Mouth – L. hand	ML

I do not think it necessary in this connection to pay attention to the very slight difference of potential produced between the two feet—nor therefore to the slight difference that is found between what I used to designate as the axial and right lateral leads according as the left or right foot is taken in conjunction with the right hand in what I now term the right inferior lead—nor the slight difference obtaining between the equatorial and left lateral leads according as the right or left foot is taken in conjunction with the left hand in the left inferior lead. In my five leads I have placed the two inferior above the two superior leads—a little reluctantly I must confess, because it offends my sense of symmetry, and I should have preferred to enumerate them in their proper historical order, but I make the concession in deference to what has now become an established custom, and in order that the first three leads of the list should correspond with Leads I, II, III in ordinary use. I do still prefer, however, to explain matters to students by comparing the less obviously contrasting couple of leads axial (II) and left lateral (III). The counterpart of left lateral is right lateral and of axial equatorial, and in talking to students the difference between right and left sides comes out much more clearly with these counterparts than with Leads II and III.

I sometimes ask my electrocardiological friends why they choose these leads, I, II, III, and the only answer I ever get is that it is the custom. Perhaps my friends enlighten me further by stating that in certain leads certain peaks are larger or smaller according as the right or left sides of the heart predominate, but no rational explanation of such statements is ever offered. I find it far simpler and more rational to think and talk about electrical effects on the right and left sides of the body than about peaks by Leads II and III. And I am sure

the distinction between "strong" and "weak" leads is helpful to any intelligent student. A line from the right side to a foot agrees with the normal tilt of the heart and the lead, right hand —foot, is favorable. A line from the left side to a foot crosses the normal tilt of the heart and the lead, left hand—foot, is unfavorable. The former is the axial or "strong" lead, the latter the equatorial or "weak" lead. And I cannot conceive how it came to pass that Einthoven, who in every other particular confirmed my first statements, contradicted this particular statement of mine with regard to the left lateral lead. The contradiction was a contradiction of principle, and was itself in contradiction with his own facts as well as with the form in which he chose to express the physical principle that the sum of electromotive forces round a given point is equal to zero. His equation II — I = III or Right — Transverse = Left is obviously correct. Put into other words the equation becomes Strong — Transverse = Weak. It was (and is) obviously absurd to demur to the statement that the right is a favorable or strong lead and the left an unfavorable or weak lead. Obviously further as the heart's axis approximates to the vertical the left approximates to equality with the right. And as the heart's axis approximates to the horizontal, the left magnitude diminishes to and beyond zero. As for the transverse lead it may be either strong or weak; strong when the heart's axis approximates to the horizontal, weak when the heart's axis approximates to the vertical.

I am restating to-day a view of the matter at which I arrived more than twenty-five years ago, and which nevertheless is still at this moment practically a novel view, yet simple and rational.

What I did in early days was to compare the normal electrical effects on the two sides of the body and to correlate them with the normal obliquity of the heart. What I am trying to do to-day is to repeat that comparison between the two sides, and to restate it in simple mathematical form. Thus, *e.g.*, while in early days I knew that the transverse effect was larger or smaller as the heart's axis was inclined more or less from a

vertical line, to-day I am able to say that "the magnitude of the transverse spike" varies with the sine of the axial angle. Thus, *e.g.*, with a heart at an angle of 30° giving a transverse spike = 0.00050 volt, the theoretical value of the spike if the angle is increased to 40° (as, *e.g.*, by rise of the diaphragm) will be 0.00064 volt.

In 1888–90 I assumed as the normal obliquity of the heart to the left an angle of 45°. This year (1913) when I had an opportunity of repeating observations upon the subject of most of my first observations, viz., upon Thomas Goswell, my former laboratory man, it was not a little interesting to find the following values of the right and left inferior spikes, viz., R = 9, L = 3, from which I am able now to make the following calculation:

$$\tan \alpha = 2\,\frac{9-3}{9+3} = 1, \quad \therefore \alpha = 45°.*$$

I completed my first review of the weak and strong leads by observations upon quadrupeds (dog, cat and horse) and upon human subjects with transposition of the viscera. I took a good deal of trouble to find cases of *situs viscerum inversus* because obviously at a time when these things were new it was of some importance to see whether there was a corresponding transposition of the electrical pulse. And I found at once in two such cases what has since then been rediscovered by a good many observers, that the electrical corresponds with the anatomical transposition. The strong leads in these rare cases, in correspondence with the fact that the heart is tilted to the right, are the right superior and the left inferior; the weak leads are the left superior and the right inferior; and the direction of the transverse effect is reversed. You may find all these facts recorded in my first publications; the diagram to which I am now pointing has been in use since 1887. So it is an old story, yet I venture to submit also a new story in so far as the full significance of the statements it contains are even now very imperfectly known and understood.

* I think, but am not sure, that projected upon Einthoven's triangle the values II = 9, III = 3 give the angle α with the horizontal = 49°.

VERTICAL AND HORIZONTAL HEARTS

These are records [not here reproduced] of two contrasting types of heart, which probably would be diagnosed by most electroclinical authorities as instances of right and left ventricular hypertrophy, but that I prefer to designate as vertical and horizontal, without of course venturing to deny that they may not as well have been instances of right and left hypertrophy.

The measurements of the ventricular spikes that we shall take into calculation are as follows:

	Transverse.	Right lateral.	Left lateral.
Case A	5	22.5	20
Case B	20	7	−17

The angle α formed with the vertical by the electrical axis of the heart is according to formula

In case A, $\tan \alpha = 2\,\dfrac{22.5-20}{22.5+20} = \dfrac{5}{42.5} = 0.12$, $\therefore \alpha = 7°$

In case B, $\tan \alpha = 2\,\dfrac{7+17}{7-17} = \dfrac{48}{-10} = -4.8$, $\quad \therefore \alpha = 180° - 78° = 102°$.

That is to say, the electrical axis is not far removed from the vertical in Case A (7° to the left) nor far removed from the horizontal (12° above) in Case B.*

It is probable from these two values that the anatomical axis of the heart must be further from the vertical and nearer to the horizontal in B than in A. And we may accordingly briefly refer to A as of a vertical and B of a horizontal type. The inference is in these cases very fairly supported by the skiagrams which exhibit in Case A a heart shadow spread out almost horizontally upon the diaphragm. There is in fact only a rough parallelism between an electrical axis as determined by calculation from left- and right-hand spikes and an anatomical axis as determined from inspection of the skiagram. Strictly speaking it is only the angular inclination of the current-axis that is determined from the electrical data not that of the anatomical axis; and although it is allowable to suppose that the angle must be diminished by protrusion of the right heart

* *i.e.*, according to the current rubric A presents small R_I, large R_{III}.
B presents large R_I, reversed R_{III}.

increased by protrusion of the left heart, there are other conditions to which variations of the electrical angle might be attributed. Thus in the two particular cases of vertical and horizontal heart quoted above, there were no signs or symptoms of either right or left hypertrophy, and the difference of angle could with some probability be regarded as due to differences in tone of the cardiac muscle. Heart A I believe to be a heart of good tone occupying a comparatively erect situation upon the diaphragm, whereas Heart B was a flabby heart sessile upon the diaphragm. As regards its physiological or physical tone, A was a hard heart, B a soft heart. I have met with both types in all sorts of people, without as well as with valvular lesion and at all ages. Type A has in my experience occurred most frequently in young active subjects, Type B in old and flabby subjects. In subjects of gross valvular lesions affecting especially the mitral valve and the right side of the heart, Type A has been the rule, whereas cases affecting especially the aortic valves and the left side of the heart, have more frequently belonged to Type B. But the rule has in my experience proved far from invariable, many apparently normal persons present well-marked electrical signs of both Types A and B, and I have met with a considerable number of clear cases of mitral and aortic disease that could not be assigned to either of the two contrasting types.

EFFECTS OF RESPIRATION

In my first account of the electrical action of the human heart I did not mention the influence of respiration upon the electrical pulse. I noticed it indeed but only as a disturbing influence rendering the direction of the pulse uncertain, and it was my practice in demonstrating the electrical pulse by the transverse lead from the two hands, to hold my breath in expiration, when the effect on the electrometer was to be seen at its best. I did not then make any closer study of these respiratory fluctuations of amplitude which as has been shown by the observations of subsequent observers—Einthoven, Kraus and Nicolai, Samojloff and others—are principally due to the rise and fall of the diaphragm which raises and lowers the heart

as if it were a lever hinged at the fixed point formed by the great vessels. In this way the angle formed with the vertical by the long axis of the heart is widened and narrowed, widened as the apex is raised with expiratory rise of the diaphragm, narrowed with its inspiratory descent. In ordinary slow, rather deep respiration, this rise and fall gives an altered angle of about 10°; the difference between extreme positions of inspiration and expiration may however be much greater, amounting to, *e.g.*, 36° in Einthoven's case of the subject Bak,* or even more, *e.g.*, 81° and 78° in the cases of the subjects J. C. W. and A. D. W. which we shall consider in a moment.

The effects of respiration are not the same in all kinds of leads and for all kinds of hearts; with the transverse lead RL they are indeed always in the direction of increased and decreased axial angle caused by rise and fall of the diaphragm. This point is so obvious as to require no further discussion. But with the right- and left-hand leads RF, LF, the respiratory alterations of amplitude vary according as the heart approximates to the two types which we have denoted as vertical and horizontal. Thus in the case of a heart of the vertical type, with a small axial angle, the right-hand spike increases with inspiratory descent of the diaphragm, and the left-hand spike decreases. Whereas with a heart of the horizontal type, with a large axial angle, the right-hand spike decreases and the left-hand spike, which is negative, increases with the inspiratory descent of the diaphragm. And of course with expiratory ascent of the diaphragm all these consequences are reversed. Now all these changes form a very perplexing burden to the memory. I for one cannot remember them although I can at some pains think them out when necessary, and a tabular memorandum like this comes in useful sometimes:

Effects of Inspiratory Descent of the Diaphragm Upon the Amplitude of the Systolic Spike V1.

Leads.	Vertical.	Horizontal.
I. Transverse	Decrease	Decrease
II. Right	Decrease	Increase
III. Left	Increase	Decrease (or negative)

* Einthoven, *Lancet*, March 20, 1912, p. 860.

Figs. 4 and 5 were intended to give records illustrating the facts stated in this table with the exception of those relating to the transverse lead, which have been omitted as being self-evident. But by an oversight the first case, that of B. O. B., which I selected as a case of vertical heart with a decreased right-hand spike in inspiration is in reality a borderland case of an oblique heart where the alteration of amplitude is comparatively ill-marked. This case serves, however, to illustrate another point, viz., that the right-hand spike (his Lead II) is in such cases much less markedly influenced by respiration than are either the left-hand or the transverse spikes. An oblique heart with an axial angle at 30° is at a critical angle where there is not much change of the right-hand spike and a relatively large change of the left-hand spike.

The measurements and calculations for these two cases are as follows:

B. O. B.—An oblique heart with a current-axis forming an angle of about 30° with the vertical:

	Exp.	Insp.
Right	27.5	24
Left	6	14

In Exp., $\tan \alpha = 2 \frac{27.5 - 6}{27.5 + 6} = 1.28$, $\therefore \alpha = 52°$

In Insp., $\tan \alpha = 2 \frac{24 - 14}{24 + 14} = 0.53$, $\therefore \alpha = 28°$

Difference $= 24°$

A. D. W.—A horizontal heart with its current-axis forming an angle of about 90° with the vertical:

	Exp.	Insp.
Right	6	9
Left	−13	−6

In Exp., $\tan \alpha = 2 \frac{6 + 13}{6 - 13} = \frac{38}{-7} = -5.4$, $\therefore \alpha = 180° - 80° = 100°$

In Insp., $\tan \alpha = 2 \frac{9 + 6}{9 - 6} = \frac{30}{3} = 10$, $\therefore \alpha = 84°$

Difference $= 16°$

I am afraid that these details concerning the varying influence of the respiratory movements upon the magnitude of the electrical effect in different leads must be rather wearisome.

I hope, however, they may turn out as less wearisome upon further reflection when it is realized how simple the problem becomes when taken in terms of right- and left-hand leads. And in conclusion of this chapter of the subject I shall ask you to consider at leisure the data and their calculation taken from a pair of representative experiments in which a left-hand spike is temporarily converted from positive to negative during extreme expiration, and from negative to positive during extreme inspiration. Here are the records [not here reproduced] of the case of J. C. W. in which the voluntary reversal from positive to negative is obvious, and of the case of A. D. W. in which there has been voluntary reversal from negative to positive. You may notice that in both these cases of reversal of the left-hand spike, the magnitude of the right-hand spike has not been much altered. Here are the measurements:

J. C. W.—Values of the left-hand spike LF during a rather deep inspiration and in the position of extreme expiration, *i.e.*, with the diaphragm at its highest possible point so that the heart's axis is moved through the largest possible angle from the vertical. Normally in this subject the left-hand spike is positive.

	Right.	Left.
In Inspiration	15	12
In Extreme Expiration	12	−15

$$\text{In Insp., } \tan \alpha = 2\,\frac{15-12}{15+12} = 0.222, \qquad \therefore \alpha = 12^\circ$$

$$\text{In Exp., } \tan \alpha = 2\,\frac{12+15}{12-15} = -18, \qquad \therefore \alpha = 93^\circ$$

A. D. W.—Values of the left-hand spike LF during a nearly complete expiration and in the position of deepest possible inspiration, *i.e.*, with the diaphragm at its lowest possible point so that the heart's axis is moved through the largest possible angle towards the vertical. Normally in this subject the left-hand spike is negative.

	Right.	Left.
In Expiration	+5	−10
In Maximum Inspiration	+9	+6

$$\text{In Exp., } \tan \alpha = 2\,\frac{5+10}{5-10} = -6, \qquad \therefore \alpha = 100^\circ$$

$$\text{In Max. Insp., } \tan \alpha = 2\,\frac{9-6}{9+6} = 0.4, \qquad \therefore \alpha = 22^\circ$$

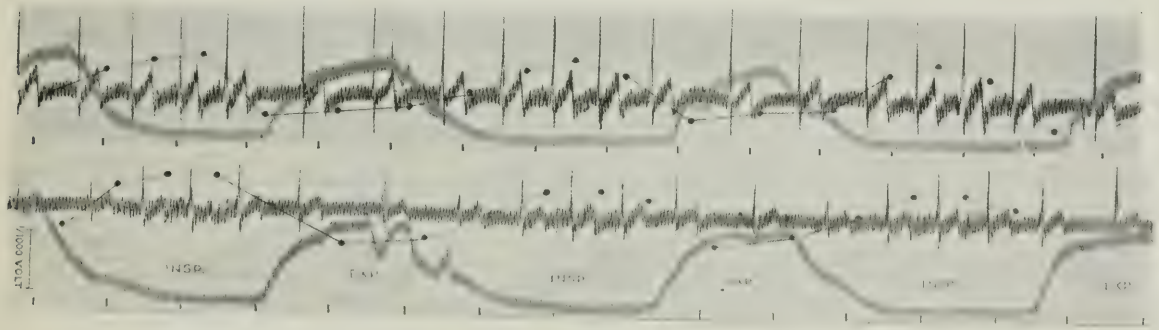

Fig. 4.—*The case of B. O. B.*—An oblique heart. Effect of respiration upon the electrocardiogram (and upon the pulse frequency) in the *right inferior* and *left inferior* leads.

In the right inferior lead (upper line) it is on the whole diminished during inspiration.

In the left inferior lead (lower line) it is distinctly increased during inspiration.

The pulse frequency in this case (as shown by the thin line between dots) is greater during inspiration than during expiration. The phases of respiration are recorded simultaneously with the electrocardiogram.

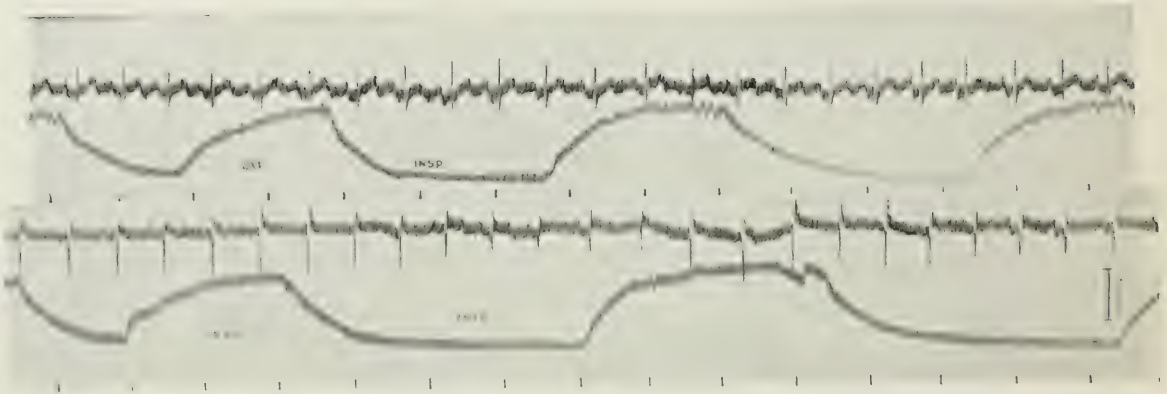

FIG. 5.—*The case of A. D. W.*—A horizontal heart. Effect of respiration upon the amplitude of the electrocardiogram in the right and left inferior leads.

In the right inferior lead (upper line) it is increased during inspiration.

In the left inferior lead (lower line) the ventricular spike is negative, and this negative spike is diminished with inspiration.

Put into graphical form the positions of the current-axis in relation to the vertical in inspiration and in expiration for these two pairs of results are as in Fig. 6. The normal average inclination of the current-axis during quiet breathing is indicated in each case by the dotted arrow.

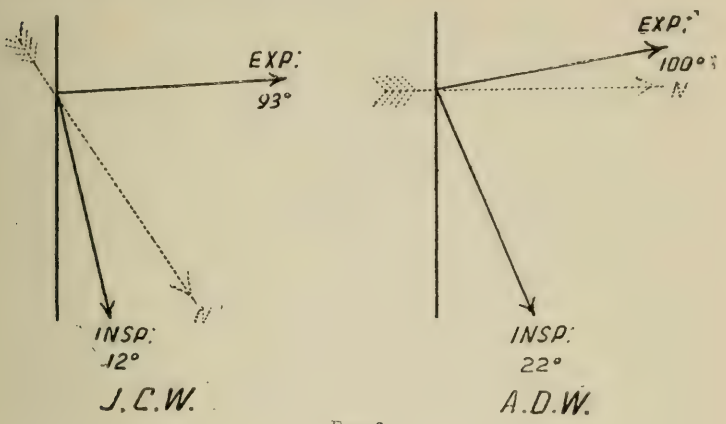

Fig. 6.

SEVERE ANÆMIA CONNECTED WITH GASTRO-INTESTINAL DISEASES*

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ALTHOUGH not aware of the frequency of pernicious anæmia in the United States, I believe that in Europe it is becoming more and more frequent. When studying the different kinds and manifestations of intestinal dyspepsia, I met with more or less severe anæmia, and will state the relationship existing between these two morbid conditions.

Combe, Addison and Biemer first described certain cases of anæmia, marked by their intensity and fatal issue, as idiopathic or pernicious anæmia. They based their reasons for separating this particular anæmia from the common type not only on the serious character of its clinical symptoms—high degree of blood changes, fever, extravasations of blood, digestive troubles, etc. —but also on the absence of any organic lesion which could be regarded as the origin of the disease and the lack of any conceivable etiology. This manner of defining a new clinical disease chiefly by negative symptoms, though corresponding to the state of science at that time, could not be satisfactory. Thus the discussion about the nature and conception of the disease began and has continued, producing numerous facts and theories, but not at all solving the problem.

One of these facts is the discovery, made by Russian and Danish authors, especially by Rosenquist, Schaumann and Talquist, that infection from bothriocephalus is able to cause a form of progressive anæmia, which by no means can be separated from Biemer's pernicious type. With that the idiopathic or primary character of the disease became doubtful. But though there were found subsequently other causes of the

* Delivered October 18, 1913.

disease, as syphilis (Fr. Müller), pregnancy (Gusserow), etc., the remaining number of unexplained cases was still large.

In interpreting these cases from 1900 to the present time, two different theories are mostly discussed. According to one of them, inaugurated by Paul Ehrlich, the characteristic sign of the disease in the megaloblastic degeneration of the blood-forming structures; the other, advocated by W. Hunter, starts from the hæmolytic nature of the disease and supposes its origin to be found in a more or less specific affection of the gastro-intestinal tube.

Ehrlich's hæmatological studies, by means of differential staining, led him to the conclusion that a distinct type of bone-marrow change, which causes the appearance of large and intensely colored, partially nucleated red corpuscles—megalocytes and megaloblasts—cell forms which under normal conditions appear only in embryonic life, is not only the essential symptom but even the cause of pernicious anæmia. He and his followers (Lazarus, Engel, Naegeli and other hæmatologists) speak of pernicious anæmia only if this sign and some other blood changes, as increased color-index, poikilocytosis, diminished number of leucocytes, with comparative increase of lymphocytes, are evident.

There can be no doubt that by accepting Ehrlich's theory the conception of the disease, given by Addison and Biemer, is changed to some extent, for clinical observation shows that megaloblastic degeneration may be present in some cases of common (secondary) anæmia and absent in others belonging to the idiopathic group. The megaloblastic type of blood formation, though a characteristic symptom of the disease, as is the pigmentation of internal organs and the tendency to hemorrhages, does in no way explain the etiology. If pigmentation is the consequence of hæmolysis, the changes in blood formation must be taken as a reaction of the bone-marrow to the unknown cause of hæmolysis (Naegeli).

Hunter was the first to perceive the hæmolytic nature of pernicious anæmia. According to him this form is distinguished from all other forms by the excessive presence of iron in the liver,

due to the more or less continuous destruction of red corpuscles within the portal circulation. Though he could not prove the presence of the hæmolytic process itself, nor produce the hæmolytic substance from the organs, he tried to confirm his theory by indirect arguments, especially by demonstrating the constant presence of glossitic lesions, which he supposed to be caused by streptococcic infection, and by finding an increased amount of aromatic sulphates and other products of intestinal putrefaction in the urine of his patients.

Hunter's observations, at first neglected in consequence of the attention generally bestowed upon Ehrlich's studies, have found valuable support through E. Grawitz and other clinicians. But his theory could not be accepted before the supposed hæmolytic principle was brought into evidence. This was done in 1907 by Tallquist in bothriocephalus anæmia, the best studied form of enterogenous anæmia. Only a small percentage of patients harboring tapeworm suffer from anæmia. Tallquist estimates the occurrence to be less than 1 per cent. Though this disease in its severer forms shows all symptoms of pernicious anæmia, the blood is by no means always megalocytic or even megaloblastic. Sometimes in the beginning there are found glossitic lesions analogous to those described by Hunter. Nitrogen metabolism is periodically altered so that increased decomposition of albuminates must be accepted (Rosenquist). The substance which causes hæmolysis is produced in the tapeworm itself, not in the healthy one, but in degenerated parts of it by an autolytic process. It is a cholesterin ester of oleic acid, which in small quantity also may be gained from different normal organs. If the bothriocephalus is removed by an efficient cure the patients may entirely recover, but in some cases there still remains an increased tendency to the recurrence of anæmia (Schaumann).

Bothriocephalus is not the only worm the presence of which gives rise to anæmia, as the hook-worm, which sucks blood from the mucous membrane, leads in this way to successive anæmia. But as it produces no toxin, the megaloblastic blood degeneration is missing. On the other hand, there have been described

some undoubted observations of the pernicious form connected with the common tapeworm (Tænia saginata) and with trichocephalus, round-worm and certain protozoan infections. Even in tropical sprue an anæmic condition of severe character is sometimes seen, although exact investigations of blood changes are not yet published.

Regarding the hæmolytic nature of bothriocephalus anæmia, it is natural to suppose a similar factor in other forms of anæmia connected with gastro-intestinal disorders. Besides the large class of unexplained idiopathic anæmias, there are two more morbid states which from this point of view excite our interest—namely, the coincidence of a small non-ulcerated stomach cancer, with progressive anæmia of the megaloblastic type, and the development of the same pernicious form in the course of cicatricial stenosis of the small intestine.

As regards the first condition, the fact is well known that the so-called cancer anæmia especially appears in cancers of the gastro-intestinal tract. Though the blood generally does not show the characteristic signs of toxic bone-marrow reaction, hæmolytic substances comparable to those derived from the bothriocephalus body have been separated from cancerous tissue by Kullmann, Tallquist and others. They may be, but seldom are, strong enough to overcome the regenerative power of the blood-forming organs. In the cases referred to, the post-mortem examination did not exhibit any other cause of the anæmia, as, for instance, inflammation of the mucous membrane, cancerous degeneration of the bone-marrow, etc. V. Noorden, Israel, Scott, Bloch, Lazarus, Nauer have observed such cases, and I can add two of my own. Shall we regard this coincidence as chance? Are there two different diseases coexistent? I think it is difficult to deny entirely any connection between them.

The second condition, cicatrized tuberculous stenosis of the small intestine, accompanied with pernicious anæmia, was first described by Faber and Borchgrevink. Most of the cases hitherto known showed no fresh ulcerations nor any notable inflammation. In my own two observations even microscopic investigation demonstrated only the same state of insignificant

lymphocytic infiltration of the mucous membrane as in other cases of pernicious anæmia. Neither the preëxisting tuberculous ulceration nor the scars, but only the irritation of the intestinal walls following the chronic retention of the contents, can be regarded as the etiologic factor.

The group of unexplained cases of pernicious anæmia in relation to gastro-intestinal troubles now claims our attention. Biemer has described certain digestive disturbances, as anorexia, gastric discomfort, weak digestion and periodic diarrhœa, as the chief symptoms of the disease called after him; but he regarded them as consequences of the anæmia, as did succeeding observers except Hunter. Hunter says:

> A history of antecedent gastric or intestinal disturbances was found in each of twenty-five cases. In no case were these of sufficient character or intensity to account for the supervention of such an intense degree of anæmia. The various disturbances connected with this tract have an important significance, not as a cause of the disease, but as symptoms of the gastric and intestinal infective lesions associated with the disease.

In other words, anatomical lesions, especially the infective glossitic changes, are the chief factors; the digestive troubles are only mild manifestations of these lesions.

According to the physiological point of view of modern clinical investigation we will first consider these functional disorders. The fact of the constant absence of hydrochloric acid in the gastric contents of all patients suffering from pernicious anæmia was somewhat neglected until Martius found out that we had to deal not only with a deficiency of hydrochloric acid, but of the whole gastric digestion, *i.e.*, with Achylia gastrica. Even this fact did not attract much attention, for by the more common use of the stomach tube it soon became evident that many persons have no gastric juice at all without suffering from any digestive trouble, and that this condition does not depend necessarily on atrophy of the gastric glands. But the conception has considerably changed. Since Oppler and Einhorn first described chronic diarrhœas following gastric achylia the different forms of gastrogenous intestinal dyspepsia have become

an object of clinical interest, the consequences of which cannot yet be fully estimated. Faber, who analyzed 207 cases of achylia, found in 22, or 11 per cent., marked signs of pernicious anæmia, and in 22 others a hæmoglobin of less than 50 per cent. While the anæmia in these latter cases only showed the character of a serious chlorosis, in the others all the blood changes of the pernicious form were present: increased color-index, poikilocytosis, leucopænia and megaloblastic degeneration of the red corpuscles. Furthermore, Faber observed a number of patients suffering from severe anæmia, whose achylia had already existed and had been proved many years before they fell sick with anæmia. This fact led him to the conclusion that pernicious anæmia is not, as generally supposed, the cause of gastric achylia, but, on the contrary, the result of it.

Coming now to the intestines, it was Grawitz who, following Hunter's suggestion, attached most weight to enterotoxic products, which he supposed to result from insufficient or faulty denaturation of the albuminates. Unfortunately, he also was unable to produce any argument which could confirm his theory. I therefore first undertook to study the frequency and kind of the intestinal troubles, mentioned by most authors as symptoms of the disease, but not yet analyzed by any one.

Among my patients suffering from idiopathic pernicious anæmia there were sixteen, the third part of all observed, who showed more or less marked signs of intestinal disorders. They mostly had periodic diarrhœa, or diarrhœa alternating with constipation. Generally the diarrhœa was of an insignificant degree, and was not accompanied with colicky pains.

When examined by means of my test-diet or a similar food, the fæces were of a pulpy consistence, and had an offensive odor, which sometimes was distinctly sour. Corresponding to that the reaction was either markedly alkaline or acid, the normal test-diet stool being nearly neutral. When ground up with water to a fluid consistence, often small flakes of mucus could be seen, whereas larger particles were constantly missing. A few times the whole mass showed a mucoid character, though no flakes could be isolated. Chemical tests showed that this condition was

caused by the presence of a large quantity of nucleoproteid, a product of the intestinal epithelium, which under normal conditions appears only in small quantity.

As to the remains of food, connective tissue was often found, due to the lack of gastric juice. Macroscopic remains of muscular tissue seldom could be seen, but often increased quantities of microscopic muscle fragments were present. Starch granules colored blue with iodine and numerous needles of fatty acid and soaps were easily demonstrated by the microscope. The fermentation test at times was positive.

Interpreting these facts we have to deal with a kind of intestinal dyspepsia, sometimes associated with an irritation of the surface epithelium and periodically bringing about a transitory inflammation of the mucous membrane. These are the same conditions as found in other non-anæmic cases of gastric achylia; they offer the real features of what is called gastrogenous dyspepsia. As in all these forms the symptoms and findings are variable and manifest themselves periodically. The stools at times may be completely normal in character. Therefore it may happen that such cases are only identified by carefully inquiring into the previous history, as the symptoms may be absent during the period of observation. In the majority of my cases transitory diarrhœa and gastric achylia, present in all my cases except one, preceded the development of the anæmia many years.

There is complete harmony between my observations and those of Faber, especially as to the pre-anæmic digestive troubles. Endeavoring to answer the question as to how achylia may lead to anæmia, Faber believed it to be by way of the intestine. According to his suggestion the lack of hydrochloric acid gives rise to the establishment of hæmolytic bacteria in the small intestine, but he did not prove either the existence or the nature of the supposed intestinal disorders. This was omitted by all the earlier authors, as, for instance, Martius, who sought the cause of pernicious anæmia in atrophy of the mucous membrane, proceeding from the stomach to the duodenum and small intestine. As to my experience, not only is the frequency of in-

testinal dyspepsia in pernicious anæmia striking, but even more so is the frequent occurrence of various degrees of anæmia in all forms of chronic intestinal dyspepsia. The percentage of serious forms of anæmia occurring in the course of gastrogenous dyspepsia is much higher than that occurring in simple achylia not connected with diarrhœa. Thus the common non-specific intestinal dyspepsia forms the missing link between achylia and pernicious anæmia.

We cannot here thoroughly discuss the theories concerning the real causes of intestinal disorders following gastric achylia. Faber's suggestion, based on the passage of pathogenic bacteria through a stomach not containing hydrochloric acid, a theory established long ago by Baumann and his pupils, is indeed useful, because the condition of chronic intestinal dyspepsia cannot be understood without the coöperation of bacterial fermentation. But surely the lack of hydrochloric acid cannot by itself entirely explain the problem. The connective tissue and other food remains which are not dissolved in the achylic stomach contents may be accused with more probability, as they are not only a lurking place, but also a good culture medium for decomposing bacteria, which under these conditions settle in the duodenum. Together with burdening of the intestine with insufficiently dissolved food, they slowly lead to a state of exhausted pancreatic digestion, which can sometimes be proved by the appearance of unchanged nuclei in the fæces.

Turning now to the anatomical lesions, the glossitic changes known as Hunter's tongue can be observed in the majority of cases. Microscopic investigation shows proliferation of the epithelial covering, alternating with atrophic spots, fissures, mild degrees of lymphocytic exudation, and other signs of chronic inflammation. Streptococcic invasion, found by Hunter, can only be regarded as accidental. It may also be noticed that irritability of the tongue, corresponding to the characteristic appearance, sometimes precedes the anæmia (Matthes).

Faber and Bloch, who studied the microscopic structure of the stomach in achylia, found the lymphatic follicles enlarged and indistinctly shaped, lymphocytes more or less spread

through the mucous membrane, and the glands diminished in number and size, doubtless signs of chronic inflammation, but no atrophy.

As to the intestine, the question introduced by Nothnagel as to whether atrophic states of larger parts of the mucous membrane, like those occasionally seen in the stomach, are in any way to be found, can be answered in the negative. Even by comparing the weight of the whole mucous membrane with that of a normal one, and by counting the number of Lieberkühn's glands in sections from different parts, Meyer and v. Lippmann in my laboratory, agreeing with Faber and Bloch, found no atrophy. There were the same signs of a light chronic inflammation of the mucous membrane as found in the stomach. Only once did I observe a fresh inflammation in the lower part of the ileum.

Gathering up these facts, we must conclude that the anatomical lesions corresponding to the functional disorders do not show any specific character, that they must be regarded rather as the result than as the cause of the digestive disturbances. Hunter's suggestion of a primary infectious origin of pernicious anæmia is proved neither by anatomical nor by bacteriological facts.

But the fact of coincidence of the gastro-intestinal lesions with the anæmic condition cannot prove their causal connection. Are there any arguments to make evident the hæmolytic nature of these lesions? Though it is well known, and can easily be demonstrated, that the blood taken from the peripheral veins in pernicious anæmia rarely shows signs of hæmolysis, such as colored serum or decreased resistance of the red corpuscles to salt solution, there are some remarkable facts supporting the theory of a hæmolytic agent circulating especially in the portal venous system. The first fact, advanced by Hunter himself, is the excessive accumulation of iron in the liver, which is more than three times as great as under normal conditions, and does not stand in a correct relation to the amount of iron in the spleen and the kidneys. The other fact, exhibited by M. B. Schmidt and communicated by Matthes, is the reddish (hæmoly-

tic) color presented only by the portal lymph-vessels and lymph-nodes in patients dying from pernicious anæmia.

Accepting the suggestion of a hæmolytic agent entering into the portal system, the question arises whether it is produced in the chyme or in the mucous membrane of the bowels. The presence of an increased amount of hæmolytic substances in the intestinal contents or in the fæces, if ever proved—as it has not been—cannot satisfy us, because the decomposition of almost all sorts of food sets free transitory traces of oleic acid. This acid is shown by Tallquist and Faust to be an efficient hæmolytic substance; the normal epithelium must be able either to withstand such a noxious substance or, if absorbed, to destroy its toxic character by changing its structure. By the same reason the researches of Lüdke and Fejes, who extracted hæmolytic substances out of the different intestinal bacteria, especially from the *Bacillus coli*, isolated from pernicious anæmia, do not explain anything, as so far there is no evidence that the bacteria penetrate the covering epithelium and grow within the mucous membrane.

On the other hand, Korschum and Morgenroth produced hæmolytic lipoids from different normal organs; for instance, from the mucous membrane of the intestine, the pancreas, etc. Starting from that fact, Berger and Tsuchiya in two of our cases of pernicious anæmia extracted, immediately after death, the mucous membrane of the whole gastro-intestinal tube. The lipoids extracted from the stomach, and even more from the small intestine, showed a hæmolytic power about ten times higher than those of normal organs. I believe this result to be of great importance, although Ewald and Friedländer could not confirm it. But they omitted to examine fresh organs.

Remembering the microscopic signs of chronic inflammation, mentioned before, we are allowed to suppose certain degenerative changes in the mucous membrane of the small intestine which may set free hæmolytic substances. It is obvious that the constant absorption of even a small quantity by an organ, especially destined for absorption, can exhaust the reproductive power of the bone-marrow.

Another fact, just stated by Kabanow in my laboratory, may confirm this suggestion. Working with the new Abderhalden method of proving the origin of diseases by searching for the so-called specific protective ferments circulating in the blood, Kabanow in three cases of pernicious anæmia found that the mucous membrane of the small intestine was regularly decomposed by the serum; that of the stomach only once; the colon, liver, etc., were never decomposed. This means that the small intestine, and to a less degree also the stomach, undergoes degeneration.

If the experiences hitherto spoken of seem to confirm the causal connection between certain digestive disturbances and the development of pernicious anæmia, I do not believe that all cases of this disease have this origin. Only one-third of the patients suffering from pernicious anæmia show marked intestinal troubles, and the number of dyspeptic persons who acquire severe anæmia is not higher. Moreover, experience shows that the anæmic condition, when once developed, does not always go parallel with the intestinal troubles. While the latter improve and relapse, the anæmia often maintains its progressive character even when the digestion has recovered by careful treatment. Schaumann studied the fate of his patients who recovered from bothriocephalus anæmia and found that part of them still inclined to relapsing anæmia. This observation and other circumstances induced him to believe that a constitutional state forms the basis upon which different lesions may give rise to pernicious anæmia. Indeed, Bartlett, Pateck, Matthes, and others saw striking examples of family or hereditary development of the disease. Pernicious anæmia can be produced also by causes which have no connection with the gastro-intestinal tube, such as lues, pregnancy, septic infection, chronic nephritis, etc., though the intestine as the chief place of absorption prevails. If the experiment newly made by Eppinger and Delcastello, that extirpation of the spleen is able to cure certain cases of pernicious anæmia, should be confirmed, we must deal with the possibility of an accumulation of the hæmo-

lytic agent in the spleen, as Banti supposes to take place in the disease called after him.

The blood changes of pernicious anæmia, interpreted by the hæmatologists as a degeneration of blood formation, leads to the same suggestion of a varied etiology of the disease. There is no proof that toxic irritation of the bone-marrow produces the megaloblastic type of the blood formation. Every sort of exhaustion can do the same. Often the first attack can be combated by stimulating the blood-forming organs with arsenic. If the cause of permanent blood dissolution is at once removed the anæmia may disappear.

It is useless to discuss a problem which cannot be solved with our present knowledge. Turning to the more practical question, Do the facts in any way influence our treatment of pernicious anæmia? Grawitz, who first accepted Hunter's theory, based his opinion more on his successes in treating the patients with a carefully composed diet and with systematic washing of the stomach and colon than on pathological investigations. We all know the difficulties of feeding these patients. The irritability of the tongue and stomach is extreme, and makes them refuse any except soft food. Regarding the constant lack of hydrochloric acid in the stomach and the intestinal dyspepsia depending on it, we can give some rules which must be observed in the diet. The first is to banish all irritating substances, such as salt, spices, too hot or too cold food, sparkling drinks, high percentage alcoholics, etc. All food must be minutely chopped or mashed because the stomach, which by its juice should assimilate the ingested material, does not functionate as it should. By the same reason all raw food, as fruits, salads, raw or smoked meat, etc., are not permitted. Whatever is eaten must be thoroughly cooked or grilled.

As to the combination of the food, I do not agree with Grawitz, who, fearing that the decomposition of albuminous material could supply the hæmolytic agent, put his patients on a vegetarian régime. Personally, I do not prefer any particular food, but adapt the diet in each case to the report of the fæces examination, which must be repeated at least every other day.

If the stool shows fermentation of carbohydrates (acid reaction, remains of starch and iodophilous bacteria, production of gas in the thermostat), all vegetables, potatoes, rice, bread, sometimes even sweets, must be avoided for some days. Later, under control of the fæces they may be allowed. On the other hand, evident putrefaction of the stool (with alkaline reaction, increased fragments of muscle-fibres, etc.) affords restriction of the albuminous food (meat, eggs, etc.), and a more farinaceous or milk diet. Changing the régime has of itself a good effect.

As achylia of the stomach is a constant symptom of pernicious anæmia, it is usual to give the patients hydrochloric acid with the meals. I sometimes exclude arsenic and other drugs, but am convinced that by regular use of hydrochloric acid not only will the intestinal disorders be diminished, but also the anæmic state can be considerably improved. Grawitz, Cottan and others have reported cases treated and cured by hydrochloric acid alone. If hydrochloric acid proves useless, I recommend systematic washing of the stomach with physiological salt solution or salicylic acid solution (1 to 1000). More efficient are insufflations of oxygen directly into the intestine by the duodenal tube. Grawitz applied lavage of the colon with 2 per cent. salt solution, and emphasized their effect. My own experience is too slight to form an opinion about them, but I believe it is better to wash the duodenum than the colon. I prefer these methods to the use of disinfecting drugs, as none of them has proved satisfactory.

As mentioned previously, the improvement of anæmia does not always keep pace with the disappearance of the intestinal disorders, yet subsequent care for the latter has a real effect also on the former. Preceding the development of anæmia, the digestive troubles should be noticed as early as possible and submitted to careful treatment. Prophylaxis is always safer than healing the fully developed disease. Here, as in other branches of internal medicine, our motto must be: *Principiis obsta.*

THE AIR AS A VEHICLE OF INFECTION*

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FROM time immemorial, and until a very recent period, the air has been considered the chief vehicle of infection. It was certainly so when I became health officer thirty years ago. In the edition of 1883 of Parke's "Hygiene," the standard sanitary text-book of the time, malaria and yellow fever were considered typical air-borne diseases. Typhoid fever was also said to be largely air-borne, as were cholera and dysentery and the diarrhœal diseases, and sewer air was alleged an important factor in their causation. Both typhus fever and Oriental plague were thought to be spread by means of a vitiated atmosphere. My immediate official predecessor, Dr. Snow, a distinguished sanitarian of the time, considered scarlet fever only slightly contagious, but due to some epidemic influence, meaning thereby some general infection of the atmosphere. The late Dr. Janeway of this city taught me that the old watercourses of New York could be traced by the excess of diphtheria in the houses above them, due to the effluvia from the damp and infected soil. The well-nigh universal confidence in the carbolized sheet before the door and the saucer of chloride of lime in the room, as barriers against contagion, illustrate how much the air has been feared.

A great change has taken place within recent years in the attitude of scientific men towards the theory of aërial infection. Many diseases formerly considered air-borne have been shown to be transmitted invariably in other ways, and in others the rôle of infection by air has been shown to be very much less important than was formerly believed. Some former theories of the mechanism of transmission by means of the air have been

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found to be untenable and some of the alleged facts of such transmission have been subjected to much criticism. Perhaps the best manner of setting forth present-day facts and ideas is to follow in a general way the course of their discovery and development.

While there have always been some careful observers, as Richard Mead in the eighteenth century and William Budd in the nineteenth, who doubted the supreme importance of aërial infection, the first effective questioning came from the laboratory. The discoveries of Pasteur and the work of Lister encouraged the current views, as it seemed that such minute forms as bacteria could readily float in the air and the difficulty of explaining how they get into the air from infected materials did not at first seem to be appreciated. It was assumed, with little thought, that they were freely given off from fæces, urine and other discharges, and particularly that they were contained in the expired breath. The investigations of Naegeli, Buchner and others, in the late seventies of the last century, demonstrating that bacteria are not given off from moist surfaces and liquids in a state of rest, were very disturbing to the old theories.

Lister had recognized the power of the respiratory passages to ensnare the bacteria of the air, though Tyndall was the first to demonstrate the fact that the expired air is sterile, a demonstration which was substantiated by many other observers. But so firmly intrenched were the ideas as to the air carriage of disease that other explanations were sought. This seemed to be especially necessary as at about this time Koch published his discovery that tuberculosis is a contagious disease caused by a bacillus. The fact that the seat of the lesion is in the lungs, in most cases, seemed to require an air-borne and respiratory origin.

The resistance of bacteria to drying, and their transmission on small particles of floating dust, were eagerly seized upon as facts supporting the theory of air infection, and the authority of Koch, who urged this as a usual mode of infection in tuberculosis, did much to encourage a decided fear of dust as a vehicle

of infection. The investigations by Cornet in 1885 on the presence of tubercle bacilli in dust greatly increased the fear of dust infection, though Cornet's experiments are really a demonstration that even so resistant and common a bacillus is far from ubiquitous.

A vast amount of work has been done to determine the resistance of different kinds of bacteria to drying and their vitality in dust. The factors affecting the life of pathogenic micro-organisms outside of the body are so numerous that it is not surprising that quite discordant results have been obtained by different observers. Certain it is that some bacteria, as those of cerebrospinal meningitis, gonorrhœa and influenza, die so quickly that their carriage on dust is practically impossible. On the other hand, the resistance to drying of tubercle, typhoid and diphtheria bacilli indicates that dust infection with these organisms is possible. On account of the mechanical obstacles in the way of explaining how substances so difficult to reduce to fine dust as fæces and sputum could be dust-borne, other modes of infection were sought for, and in 1897 Flügge showed that infecting bacteria could be carried in the fine droplets of saliva which are thrown from the mouth during loud talking or coughing, but that expired air is sterile during quiet respiration. It had previously been shown that bacteria-holding droplets could be thrown into sewer air by the agitation of sewage and this experimental work had perhaps suggested that similar droplets of saliva might be a means of spreading disease from person to person. The followers of both Cornet and Flügge inferred much more from the work of these investigators than was warranted, or than was claimed by the authors themselves. Because Cornet's guinea pigs were infected by clouds of tuberculous dust, and Flügge's animals contracted tuberculosis when held a short distance in front of a coughing consumptive, one is not warranted in assuming that either dust or droplets are, under natural conditions, the chief mode of infection in tuberculosis, and there is still less warrant for such an assumption for other diseases. There is even less

reason for assuming that because bacteria are observed to fall on agar plates from the air of a room, the air is infectious; and there is no reason at all for the assumption that because a few germs of diphtheria or tuberculosis survive drying for three or four weeks, diphtheria and tuberculosis are dust-borne diseases.

The question of dosage in causing disease is an all-important one, though it has usually been neglected in bacteriological work on aërial infection. Winslow, however, appears to have fully recognized the importance of quantitative work, and his careful and extensive experiments on sewer air, and on the dust and air of schools and dwellings, have served to explain much of the contradictory work of others and to bring bacteriological and clinical observation in accord. Winslow's first quantitative work was on sewer air. In 1907 careful experiments were made by him in this country and by Horrocks at Gibraltar. The latter succeeded in recovering colon and also typhoid bacilli from the soil-pipes of the barracks under quite natural conditions and, though the number was not determined, he was believed by many to have thus demonstrated the dangerous character of sewer air. The number of bacteria in sewer air found by Winslow was so small that he deemed it necessary to make further observations. In nearly 200 litre samples of air from the soil-pipes of various buildings in Boston, he found sewage bacteria only four times, and only when there was splashing of sewage at the place and moment of examination. In such air as would be likely to escape from a drainage system, either from the vent-pipe or from an opening into a house, such bacteria were never found. Under ordinary circumstances *pathogenic* bacteria, such as the typhoid or dysentery bacillus, must be far less numerous than are colon bacilli. The entire absence of the latter from 193 litres of sewer air, taken elsewhere than in the immediate vicinity of splashing, shows how slight must be the danger from this source. Perhaps this point can be best emphasized by quoting from Winslow's report:

"In a surface water of good quality, like that of New York City, the colon bacillus can almost invariably be isolated from ten cubic centimetres. This means a slight degree of intestinal pollution, but experience has shown that the chance of infection from such a water is but slight; and we drink it without serious alarm. If one were to breathe for 24 hours the undiluted air of a house-drainage system, at any point not immediately infected by mechanical splashing, it appears that less than fifty intestinal bacteria would be taken in; for the daily consumption of air is about 10,000 litres, and in 200 litres I obtained negative results from air of this sort. In drinking New York water twice as many colon bacilli are ingested every day, for 1000 centimetres is a small amount for daily consumption. So there would be less danger of contracting disease from continually breathing the air of a vent-pipe, or of a soil-pipe, except where liquid is actually splashing, than from drinking New York water."

Later, experiments were made by Winslow and Robinson to determine the extent to which general air infection in an apartment is caused by droplet infection. Out of 140 litres of air taken at various points in the room immediately after ten to fifty minutes' loud speaking by a person whose mouth was infected with *B. prodigiosus,* the bacillus was found seven times. Of 74 litres examined for *Streptococcus salivarius,* none were found to contain this normal inhabitant of the mouth. The authors consider that an artificial infection of the mouth may give too high an index of air contamination, while the normal germs of the mouth may be thrown off in smaller numbers than are the disease germs from sick persons. The authors conclude that these experiments furnish "no basis for a belief that tuberculosis or any other disease is contracted to an appreciable extent through the inspired air," and are "in harmony with the conviction now generally gaining ground that aërial infection of any sort is a minor factor in the spread of zymotic disease."

Still more recently Winslow has made quantitative studies of acid-forming streptococci in New York City schools. He says:

"It is well established that acid-forming streptococci are among the most abundant forms in the human mouth, while they are absent from sources which have not recently been exposed to human or animal pollution. The numbers of these organisms in schoolroom air were found by Professor Baskerville and myself in preliminary experiments

a year ago to be quite small. We then found among 30,000 colonies isolated from 750 plates, exposed in schools with window ventilation, only ten mouth streptococci.

"In the present study in the examination of a total of 868 cubic feet of air, we found 52 mouth streptococci, or six for every 100 cubic feet of air.

"A child breathes less than 100 cubic feet of air during an average school period and these mouth streptococci must, of course, be far more abundant than pathogenic forms. At a rate of four or five mouth streptococci per day the chance of ingesting pathogenic bacteria from the air is seen to be a very slender one."

Although Winslow found so few of the bacteria of human saliva in the air of schoolrooms, on another occasion in various schools in this city he found them in dust in enormous numbers, even up to 100,000 in a single gramme. This shows that the presence of germs in indoor dust, representing, as it does, long continued precipitation, is no indication of the number floating in the air and it indicates the worthlessness of the deductions which have often been made as to the danger of air-borne infection, from the mere demonstration of the presence of disease bacteria in dust.

While bacteriology has given us facts instead of theory as to disease causation and has made a science of sanitation, bacteriology alone cannot solve all our problems. The study of micro-organisms, pathological findings, animal experiment and particularly epidemiological observations, must all be utilized and harmonized to solve the problems of disease transmission. It is for us this evening to trace the effect which modern research has had on the old belief that practically all the infectious diseases are air-borne.

Largely owing to the great authority of Murchison, it came to be believed, shortly after the middle of the last century, that the chief cause of typhoid fever was the effluvia from decomposing animal matter. When the late King Edward VII, then Prince of Wales, contracted a severe attack of this disease at Sandringham in 1871, the chance escape of sewer gas into his apartments was by every one considered a sufficient explanation of the case. Like others, I was at first possessed by the

sewer-gas bogie, but failure to connect cases with this alleged cause began to undermine my faith. The discovery of the typhoid bacillus, and more careful epidemiological study, soon determined that the greater outbreaks were usually due to infected water, milk, or other foods, and it came to be seen that the smaller groups, and isolated cases, could best be explained by contact infection. Finally, the " carrier " has been shown to be the link between hitherto unconnected cases. No form of air infection except dust has been, for many years, alleged as a cause of typhoid fever, and the reports of outbreaks of dust-borne typhoid fever in the Spanish and Boer wars and in India are based on the flimsiest evidence.

It is a common experience that typhoid fever is freely treated in the general wards of a hospital and that it very rarely indeed extends to other patients, although these patients are, perhaps for weeks at a time, breathing the air of the ward. It is not so very rare for the nurses of these typhoid cases to contract the disease, though with the development of aseptic nursing such infections are much less frequent than formerly. The only inference is that this disease is not air-borne in hospital wards but is spread solely by contact.

Cholera, dysentery and diarrhœa were, with typhoid fever, believed to be spread largely by means of air polluted by the discharges of patients. The discovery and study of the micro-organisms causing these diseases, and modern epidemiological and preventive work, have shown the part played by water, food, contact, flies and carriers, and little room is left for the action of air infection. All of these diseases can be treated in general hospitals freely, without danger of extension to other patients. It has occasionally been alleged, especially in England, that summer diarrhœa may be dust-borne, but nothing more than the seasonal distribution, in the drier months, can be adduced as evidence. Laboratory work, when conducted along quantitative lines, as carried on by Winslow, bedside observations and broad epidemiological studies, are all in accord in denying to the air any part in the extension of this important

group of excrement-borne diseases which only a few years ago we believed might be caused by a whiff of sewer air.

One of the most interesting changes which has taken place in our conception of the part played by the air as a bearer of infection concerns aseptic surgery. When one considers the origin of modern surgery, one is not surprised at the emphasis which was at first placed upon air-borne infection. The researches of Schwann, Pasteur, Tyndall and others on spontaneous generation, putrefaction and fermentation largely consisted of experiments showing that when micro-organisms floating in the air are excluded, putrefaction and fermentation do not take place. The admission of almost any air was found to give rise to the growth of bacteria and the occurrence of changes in the liquids under observation.

We are all familiar with what wonderful intuition Lister perceived the likeness of sepsis and suppuration to putrefaction and fermentation. His assumption that the former processes are due to the action of bacteria was, after the course of many years, abundantly verified. Lister's brilliant success in preventing sepsis and suppuration in wounds by destroying the infecting bacteria *in situ*, or preventing their ingress, was truly marvellous and could only have been accomplished by a man of surpassing imagination and inventive genius. Not only has the principle on which he worked been established, but much of the detail worked out by him remains fundamental in surgical practice to-day. Perhaps the greatest change concerns our view as to the infection of wounds from the air.

In the beginning the air was considered the chief source of infection. This was perfectly natural and reasonable in the state of knowledge at the time. The work of Pasteur and Tyndall had shown the ubiquity of putrefactive bacteria and their almost universal presence in the air. Nothing was known of the great variety of bacterial species, or of the numerous influences hostile to bacterial life, or of the difference between spores and vegetative forms, or of the great differences in the resisting power of different species. It had been demonstrated that the admission of ordinary air to the test-tube always

caused putrefaction; what was more natural than to assume that the admission of air to wounds carried with it the germs of suppuration and that its exclusion was a necessity?

The paramount importance attributed to air infection in surgery is well illustrated by an incident which I recall from my interne days at Bellevue Hospital. The first strictly aseptic operation in the wards to which I was assigned was to be undertaken. The room was washed with carbolic acid; the dressings and instruments were sterilized, as were the body of the patient and the hands of the operator; caps and gowns were worn by all. What was considered perhaps the chief antiseptic service was to be performed by the interne, who arose at an early hour of the summer morning to start the carbolic spray which was to disinfect the atmosphere; the spray was kept going until the end of the operation. During the operation the surgeon dropped his knife on the floor. He picked it up, wiped it on his gown and continued his work. The incident attracted little notice and caused no comment. Such was the relative importance attributed to contact and air infection in those days. To-day, I think Ochsner reflects the best surgical opinion when he says: "Air infection is not impossible, but practically no wound infection is to be considered except from contact." It is true that most surgeons, at least during abdominal operations, guard against infection by mouth spray, but infection by visible droplets, from every practical point of view, resembles infection by direct contact, and is entirely different from the general infection of an apartment by invisible floating droplets.

Although effort is made to have the air of the operating-room, as all else in it, as clean as possible, surgeons do not hesitate to operate, and do operate with success, in the air of the tenement, which is far from dust free. In fact the air of even the operating-room does contain pus germs. Many observations have been made on the presence of pus-forming bacteria in such situations, and they are practically always found to be present, appearing, often in very considerable numbers, on the agar plates exposed for their collection. Thus Harrington found in Boston operating-rooms a maximum of 131

colonies per square inch per hour. That such a number of bacteria are but rarely, if ever, capable of infecting a wound doubtless seems surprising to many. That they do not is due, doubtless, partly to the fact that, though still capable of growing on culture medium, their virulence is diminished by drying and exposure to light, and partly to their small number. That the chance of infection depends upon the dosage is now an established fact, shown both by animal experiment and clinical observation. That a single germ will cause disease is a myth of the early days of bacteriology. Many, many germs are usually necessary, the number doubtless varying greatly with their virulence, with the species, with the nature of the disease process, and with the mode of infection. That numerous living pus-forming bacteria floating in the air fail to infect when falling on the fertile soil of freshly cut tissues or exposed peritoneum, should make us scrutinize closely the claim often made that pathogenic bacteria, more sparsely distributed still, can run the gauntlet of the respiratory passages and penetrate mucous surfaces, or reach and infect pulmonary alveoli, or pass thence into the blood stream. It is not the mere presence of germs but their quantity which counts.

Fifteen years ago malaria was considered a perfect example of an exclusively air-borne infectious disease, and the virus of yellow fever was thought to be spread chiefly in the same way. It needs no reference of mine to call to your minds the brilliant work of Manson, Ross, Celli, Reid, Carroll and Gorgas which removed these from the list of air-borne diseases, and, neglecting all means of infection but the mosquito, stamped out these scourges in their strongholds.

Most of you have doubtless seen the tablet in Bellevue Hospital on which are inscribed the names of the six internes who died of typhus fever in the outbreak of 1863–1864. Of twenty-one members of the staff sixteen contracted the disease—an impressive record of its contagiousness in those days. That this contagiousness was due to the concentration of the virus in the atmosphere of the hospital was the universal belief of fifty years ago. We can, to-day, scarcely appreciate the hero-

ism of the young men of those days, who, certain in their own minds of the infection of the air, without thought of themselves, breathed it all day long in the wards while ministering to the afflicted. Until within a few short years this belief prevailed, for we find typhus fever classed as an air-borne disease by Ker in 1909, in what is, perhaps, the best text-book we have on the infectious diseases. Nevertheless, careful observers had begun to note that typhus fever is not so readily transmitted in modern hospitals as it was in crowded prisons and ships or in the Bellevue Hospital of old days. Thus 600 cases were treated in the City Hospital, Liverpool, without a single case of hospital infection. Wilder says that in the American Hospital in Mexico 144 cases were treated without transfer of the disease. Hay in Aberdeen, while noting instances of infection in the hospital during the outbreak of typhus in 1906, says that they were very few, as compared with former times, and confined to those who were brought in contact with the patients before or at entrance into the hospital. Those who handled the patients after they were cleansed escaped.

Now, thanks to the work of Nicolle, Ricketts and Wilder, and Anderson and Goldberger, carried on with such danger and at such a sacrifice, we have been given what is probably the true explanation of the epidemiological phenomena of this disease. It has been conclusively shown by their experiments that the body louse can, under ordinary conditions, transmit the disease from man to monkey and from monkey to monkey. Three of these men contracted the disease, and Ricketts died. Goldberger's attack came on five days after he was bitten by a presumably infected louse. The seasonal, geographical and social distribution of typhus fever all accord well with the theory that it is transmitted by lice, as does also its decrease in recent years in countries of the most refinement, while it still lingers in others. Formerly our hospitals, as well as our prisons and camps, were vermin infested. No wonder that typhus fever spread in Bellevue Hospital when it could be said that some of the chronic patients had not had a change of bedding in three months, while between 1881 and 1893, after the

advent of the trained nurse, 1897 cases were received in the hospitals of this city with extension to only one of the staff. Surely typhus fever must now be removed from the list of air-borne diseases.

A wider infection of the atmosphere has been claimed for smallpox than for any other disease. This claim was supported by what seemed very strong evidence, and the Local Government Board of England, the weightiest sanitary authority of the times, crystallized the then current views in the dictum that hospitals for this disease should be located at least a mile from inhabited areas. If the aërial infection of smallpox was effective at such a distance, it is no wonder that it was argued from analogy that other diseases are easily and commonly spread through the air of the sick room or the hospital ward. It is therefore important to question somewhat in detail the evidence in support of this view. This I have elsewhere done ("Sources and Modes of Infection," second edition, New York, 1912, p. 260) and will now merely summarize what I have there written. Power's report on smallpox around the Fulham Hospital in London was the immediate cause of the stand taken by the Local Government Board. Power attempted to show that whenever the hospital was occupied by acute cases the disease developed in the neighborhood, showing a progressive decrease as the distance from the hospital increased. A similar increase around other hospitals in London and in a number of English cities, and extension from the isolation ships in the Thames, has been alleged. On the other hand, the disease did not always thus spread, even from Fulham, and numerous observers in both England and this country have failed to find any evidence of such extension.

Much of the material published by the English advocates of aërial infection does not stand criticism and analysis. Even at Fulham the thickly populated streets nearest the hospital were not much affected. Other sources of infection for the cases alleged to be due to aërial infection were not excluded, and a further study of the 41 cases nearest the hospital at Fulham showed that 20 were due to direct contact. In Liverpool in

1902–1903, according to the Government inspectors, the hospitals were the sources of much air-borne smallpox, but Hope, the able health officer of the city, completely riddles their evidence. Government inspectors also claimed aërial convection at Gateshead in 1903–1904, but the local health officer showed that of the 56 cases around the hospital, on which this conclusion was based, 52 were clearly traced to contact with other cases. As for the ships in the Thames, smallpox appeared on the shore nearest the ships, and then gradually extended to a distance of two or three miles. This sort of an extension is just what would be expected in contact outbreaks. If air-borne, the near and distant communities would have been affected at the same time. It was even claimed by Dr. Thresh that the influence of the ships could be noted at a distance of four or five miles. It was also claimed that crews of vessels anchored near the hospital ships developed smallpox twelve days later. That ships leaving London during the period of the extensive outbreak in that city should occasionally carry smallpox with them is not remarkable. Finally, it was admitted that surreptitious communication with the ships occasionally occurred.

At the time of Power's investigation the control of smallpox was very lax. It is stated that many cases of smallpox walked to the London hospitals for admission, ambulance drivers stopped at public houses, children of the neighborhood rode on the steps and friends with the patient inside the ambulance. Direct communication with the hospital often occurred. It would be most remarkable, if the disease extends from, say, one hundred cases to the distance of a mile with sufficient intensity to infect many persons, that it should not extend one hundred feet from ten cases or even from one case. Why should we not expect aërial infection frequently to operate at short distances from single cases? Yet such transmission does not occur unless it be with great rarity. How rare it is for any claim to be made that this disease has been carried across the street from house to house, and how unique a rigid demonstration of such an occurrence would be! How often a single case in a crowded lodging-house, ship's steerage, or hospital

ward, fails to infect others! Yet we are asked to believe that a group of hospital patients can give rise to a whole circle of cases a half mile away. This theory demands that the amount of virus must be intensified by the number of patients, or it must under hospital conditions develop in some marvellous way outside of the body.

Collie ("Smallpox and Its Diffusion," Bristol, 1912), who served in five of the London hospitals, has recently reviewed the evidence derived from them, particularly that from the Homerton Hospital. He comes to precisely the same conclusion as the present writer as to the fallacy of the theory of aërial transmission. He shows that the disease was not distributed around the hospitals as alleged, that much of it was due to contact infection, that the hospitals are in locations where the disease is rife, that it was often prevalent before the hospitals were opened, that unreported mild cases were very numerous, that the administration of the hospitals was lax and that the disease, as a consequence, was spread from them by personal contact. Collie furthermore shows that the incidence of smallpox in institutions adoining the Homerton Hospital has been small, very different from what is required by the theory of aërial convection, and he gives figures to show a very small incidence of the disease on other fever patients treated in adjoining wards of the same hospital though a large number of these were unprotected by vaccination.

With the uncertainty as to the true nature of Oriental plague, it is not surprising that, among other theories as to its mode of infection, aërial transmission held a prominent place. The development and demonstration of the theory that plague is primarily a disease of rodents, and that it is spread to man almost exclusively through the agency of fleas, is a fine example of modern scientific method. In this work both the epidemiologist and the laboratory man played important parts. The most complete and convincing evidence was that furnished by the last English Plague Commission in India. Some of their experiments, demonstrating the agency of fleas in the propagation of bubonic plague, also demonstrated conclusively

the entire absence of air-borne infection. Many experiments were made in infected native villages, in houses in which plague had been rife, and from which the human inhabitants had been removed. Numerous rodents were exposed in flea-proof wire cages, or protected by tangle-foot, but of course freely exposed to the air of the apartments. As long as fleas could not reach the animals they never developed the disease, but numerous control animals exposed in the same places, but not protected from fleas, became infested with these insects and contracted plague. These experiments are entirely in accord with the experience that cases of bubonic plague may be treated in general hospitals without danger, if only the place is kept free from vermin. At the present time probably all epidemiologists are agreed that air infection plays no part in the spread of the disease.

With pneumonic plague, conditions are very different. This form of the disease is usually highly contagious from person to person. The pulmonary secretion is large in amount and crowded with bacilli. Where this type of disease prevails, unsanitary habits prevail also, and the opportunities for contact infection are great. Nevertheless the contagiousness of the disease is so great that, in the especially virulent form which recently occurred in Manchuria, many observers were led to believe that the disease was easily air-borne, presumably by droplets. The primary focus of infection in a large proportion of cases seemed to be in the larger bronchi, though sometimes it was in the tonsils. Strong and Teague sought to demonstrate the presence of droplets by exposing agar plates held vertically before the patients, or in some instances guinea pigs were so placed. It was found that no plague bacilli were deposited on the plates during quiet, or even labored breathing, though held within a few inches of the face. When held before coughing patients, even at the distance of a metre they were commonly infected. The wards were crowded with patients and, owing, it was believed, to the droplets, the care of the patients proved very dangerous for the attendants. This danger seemed to be greatly reduced by wearing masks to prevent breathing the

germs. The weather was extremely cold and damp, so that the breath was visible and often condensed on the agar plates. Teague and Barker have shown that plague bacilli in droplets, sprayed from an atomizer, die in a few minutes in dry warm air, but live much longer in cold damp air, which fact he suggests may account for the occurrence of most outbreaks of pneumonic plague in the winter months. While it would appear that the air in the hospital wards where these observations were made was dangerously loaded with bacilli-bearing droplets, it may be that it would be quite different if cases of this disease were treated in a ward with sufficient air space and a distance of, say, six feet between the beds. It may be that persons in such a ward would be in no greater danger of contracting plague, unless they approached close to the patient, than they would be of contracting diphtheria in a modern diphtheria ward, and this we shall see is very slight.

The discovery of the etiology of Mediterranean fever is another brilliant example of the effectiveness of modern scientific methods. In the multiplicity of theories resulting from ignorance, of course aërial transmission had its place. Even two or three years after Horrocks' successful work I met a highly educated priest from Malta who insisted that goat's milk had nothing to do with the disease, but that it came from poor drainage and the resulting effluvia. Among Horrocks' numerous experiments were some in which he showed that the disease could be given to monkeys by making them inhale dust which had been artificially infected with large numbers of *Micrococcus melitensis*. Experiments such as this, under unnatural conditions, have little value. But when Horrocks kept monkeys under natural conditions, in separate cages, close by infected animals, they never developed the disease. There was then no air-borne infection. The coccus of this disease has a considerable resistance to drying, much like that of the typhoid bacillus, and theoretically this might therefore be assumed to be a dust-borne disease. The presence of the germs in the urine of numerous goats which move freely about in the

streets lends color to this theory. Horrocks showed that it can be dust-borne but under natural conditions is not.

That influenza is air-borne, even over long stretches of country, was a common theory twenty years ago. That the air has no part in its extension from place to place, but that its presence is always due to the importation of cases, was abundantly proved by the observations of Schmid, Parsons and Leichtenstern. That the disease may be spread within a few feet of a coughing and sneezing patient by means of visible droplets is highly probable. That it is transmitted by floating droplets, or by dust, is not likely, as the bacillus has a low resisting power. Reliable clinical observations as to its extension in hospitals and dwellings appear to be lacking.

Judging from the feeble resistance to drying of the coccus of epidemic meningitis, that disease seems unlikely to be air-borne and certainly cannot be dust-borne. The fact that meningitis treated in open wards does not spread also contraindicates it, though the high proportion of carriers to cases somewhat weakens the argument.

Rather scanty experimental work indicates that the virus of epidemic poliomyelitis may possibly be dust-borne and the possibility of its transmission on house flies, by biting flies and by direct contact with infected secretions, has been demonstrated; but which mode of infection actually prevails, under natural conditions, we do not know. Because the disease occurs chiefly in dusty weather is no more reason for assuming it to be dust-borne than is the fact that it prevails in fly time proof of its transmission by flies. As in meningitis, absence of infection in hospitals indicates that the air is not an important vehicle of infection.

Although much is said, particularly in semi-popular literature, about the aërial transmission of pneumonia, we really know nothing of how the coccus of this disease reaches the lungs. Nearly half the population at times carry the germs in the mouth. Pneumonia develops only when bodily resistance is weakened. How the coccus in these cases passes from the mouth to the lungs we do not know. That the more virulent

strains sometimes pass from person to person, causing connected series of cases, seems certain, but neither pathology, experiment, nor clinical observation has as yet shown the mode of transference.

If we regard their frequency and the demands which they make on the practising physician, the health officer and our hospitals, the most important of the contagious diseases, in this climate, are scarlet fever, diphtheria, measles and whooping-cough. Unfortunately, the laboratory has as yet been able to furnish little evidence as to the mode of their infection. A great deal of evidence has, however, been furnished in recent years by clinical observations, particularly in hospitals. Within the last dozen years the great importance of contact infection has come to be recognized, and hospital men have been learning to guard against the spread of disease in their wards by a technic similar to that made use of by surgeons to prevent the infection of wounds. There have been developed medical aseptic methods which so effectually eliminate contact infection that the part played by other modes of transmission can more easily be determined. The impression has, slowly, but so surely, been made upon hospital superintendents that air-borne infection is of small moment, that they have dared, and have found it convenient, to care for different contagious diseases in rooms opening into a common corridor, or in rooms only partially partitioned, the so-called cubicles, or even in one open ward. In the latter case the patients are said to be "barriered," which simply means that there is a card or other indication on the bed that the nurse must take special care in passing from patient to patient. There has resulted a considerable mass of carefullly collected data as to the aërial transmission of these diseases.

Scarlet fever and diphtheria have very generally been believed to be air-borne, especially scarlet fever. Great fear is always manifested by a neighborhood in which it is proposed to locate a hospital for these diseases and until quite recently this fear has been shared by health officers. My own ideas as to the aërial transmission of these diseases began to be greatly modi-

fied many years ago when I noticed that they do not spread from tenement to tenement in the same house unless there is direct contact. In Providence both scarlet fever and diphtheria spread to other families in about 7 per cent. of the cases, but a careful study of these extensions shows that they take place, almost always, either before the disease is recognized or because of direct contact afterwards.

After our hospital was established it was carefully watched, but no especial incidence of these diseases was noticed in the well-populated tenements distant 150 feet. The most careful study of this alleged influence of the hospital on the neighborhood was made in Boston, where it was shown that, during the period of the observation, no cases occurred within one-eighth of a mile, while in the next eighth of a mile circle there happened to be 68 cases. Similar observations have been made in other cities and in no place is any evidence forthcoming of extension even between different buildings of the hospital.

As regards diphtheria there is most convincing evidence that it is not air-borne even indoors. Although diphtheria carriers are almost constantly " barriered " in the scarlet fever building in Providence, and not rarely clinical diphtheria as well, there have been only three cases of cross infection among about 500 other patients.

In the isolation wards, where different diseases are all cared for in rooms opening into a common corridor, with 126 cases of diphtheria and 130 " carriers," there has been no extension to the 541 other patients. Rundle, in Liverpool, had no extension from 42 cases to 699 others in his open ward. Thomson had only two cross infections in over 1200 cases. All of the English hospital superintendents agree that this disease is not air-borne. The extremely small number of cross infections are admitted to be due to failure in aseptic technic. It is also worthy of note that many hundreds of students have passed through the diphtheria wards in Philadelphia without contracting the disease. Although diphtheria is not air-borne, the bacilli are among the more resistant bacteria, more resistant than tubercle, influenza, or pest bacilli, or the cocci of menin-

gitis or pneumonia or the cholera spirillum, and we should expect diphtheria to be more readily air-borne than the other diseases mentioned. Teague found the bacilli on a third of all plates exposed in front of coughing patients. The clinical evidence that diphtheria is not an air-borne disease, when purely bacteriological evidence indicates that it is likely to be such, should make us suspicious of other bacteriological deductions—deductions which are likely to be misleading if the quantity of the dose is not critically studied.

The evidence also is very convincing that scarlet fever is not air-borne. One reason why we have believed in the air-carriage of scarlet fever is because we believed the desquamating epidermis to be infectious. This we now know is not true. For the best evidence we must again turn to the hospitals where contact infection is reduced to a low limit by aseptic nursing, but where there is no air separation. At the Pasteur Hospital in Paris there has not been a cross infection of scarlet fever among thousands of patients. None occurred in the open wards at Manchester. Thomson had five cross infections in 1290 patients in cubicles and seven among 660 patients in separate rooms, though the circulation of air in the latter was less than in the cubicles. Rundle had two cross infections and in both there was contact infection. Caiger, Goodall, Gordon, Peters, Grookshank, Biernacki and other English observers, agree that scarlet fever is not air-borne in hospitals. In Providence we have not been so successful, as we have had eight cross infections among the 582 non-scarlet fever cases in our isolation wards. That these were due to contact infection owing to failure in nursing technic or to the carelessness of internes, the superintendent, Dr. Richardson, believes. Schamberg says that none of the nearly 2000 students visiting the scarlet fever wards in Philadelphia have contracted the disease. Ward maids who do not touch patients rarely contract scarlet fever, while nurses and physicians coming within the range of visible droplets and coughed-up masses of sputum not infrequently do contract scarlet fever and diphtheria as well. In private families, maids who do not have the care of the children practically

never contract scarlet fever from them, while the parents and nurses do.

The French seem to have been the first to question whether measles is chiefly air-borne. The rapid spread of this disease in all institutions for young children where it happens to be introduced affords one of the difficult problems of institution management. Grancher, believing that the disease is largely spread by contact, attempted to control it in the wards of the children's hospital in Paris, by means of aseptic nursing, without removing the patient from the ward, and he came to the conclusion that the disease is not air-borne, as did also Rohmer at Cologne.

At the Providence City Hospital, measles cases are cared for, together with other diseases, in rooms opening on to a common corridor. There is no attempt at air separation and different diseases are attended by the same nurses and physicians. To October 1st of this year there have been admitted 320 cases of measles and 669 persons suffering from other diseases. During most of the time measles was present. During the first nineteen months, 38 cases were admitted with only one cross infection. Then we had three outbreaks, causing in all 23 cases, but since May, 1912, there have been but two cross infections derived from the 139 cases of measles cared for in this building. The fact that during many months numbers of measles cases have been treated with no cross infection, although there is no air separation, suggests that air infection does not readily occur. Then, too, when cross infections do develop they are quite as likely to be in distant rooms as in opposite ones. To us it seems, not that the virus of measles is air-borne to a distance, but that it is less easily washed from the hands of nurses and physicians than is that of scarlet fever and diphtheria. We admit a failure of our aspetic methods as hitherto practised, rather than aërial transmission. There is difference of opinion among the English hospital superintendents, and while Rundle has successfully cared for measles cases in open wards with susceptible children without cross infection, the others believe that at times the disease is air-borne. Goodall refers to

one instance where a measles case remaining over night in a ward of twenty beds caused nine cases, but he does not say how rigidly contact could be excluded. The evidence as to the aërial transmission of measles is conflicting, so that we are not justified at the present time in coming to a definite conclusion. I am inclined from my own experience to the view that it is not air-borne.

A comparatively small number of cases of whooping-cough are admitted to hospitals employing aseptic methods, so that few data are at hand as to the mode of transmission. Rundle had no extension from 13 acute cases treated in his open ward. Caiger thinks that it is not readily air-borne and Biernacki finds that cases can be aseptically nursed provided the beds are twelve feet apart. At Providence 25 cases have been treated in the isolation rooms and some cases in other wards without transfer. The only extension in the hospital has been from unrecognized cases among diphtheria patients where there was direct contact. Richardson is strongly of the opinion that the disease is not air-borne.

There is also difference of opinion as to the aërial transmission of chicken-pox in hospital wards. Most of the English observers think that at times it may be so carried. Goodall and Rundle think otherwise and their opinioin is shared by Richardson in Providence.

Caiger does not think that rubella is easily air-borne for he received 82 cases in his cubicles during eighteen months, and for several months there were from six to eight cases in the wards at a time, yet there were only two cross infections. In the isolation wards of the hospital in Providence 37 cases have been admitted with no cross infection among nearly a thousand other patients. At one time three cases developed in the diphtheria ward where they had been cared for by a nurse who was coming down with the disease.

As tuberculosis has its chief and apparently primary seat in the lungs, it was only natural to assume that the route of infection is by the inspired air. Koch's studies on the resistance of the bacillus and its survival in dust greatly encouraged this

view, as did the extensive observations of Cornet as to the presence of the germs in dust obtained in the neighborhood of careless consumptives. Then came Flügge, who attempted to show that dust is of little moment, but that infection by floating droplets is the chief mode of infection. The somewhat active controversy between the followers of these two men has, I think, merely served to convince most of us that under the conditions of the experiments both dust and droplets are capable of causing the disease. In tuberculosis the pathologists take a prominent part in the discussion of causation, but are at variance as to where the primary lesion is to be found and whether the bacillus enters by the respiratory or alimentary tract. Many think that most tuberculous infection dates back to early life and not a few derive it from the ingestion of milk, though this latter view seems to be losing ground. The weight of evidence derived from these lines of study seems to indicate that the infection is commonly, if not usually, air-borne and that the primary lesion is in the respiratory tract. There are not a few, however, who hold that contact infection plays a prominent, if not the larger part, in the distribution of the disease and that bacilli-holding sputum carried to the mouth and nose in countless ways reaches the lungs by insufflation or by absorption through the tonsils or through lower sections of the alimentary tract.

Tuberculosis is such a chronic disease that it has thus far been impossible to devise observations on human beings which would throw much light on the route of infection. Recourse must be had to animal experiments and most of these have been made under conditions far from natural. Still, a good many experiments have consisted in exposing test animals in cages in apartments occupied by consumptive animals or human beings. A large number of tests made by the Department of Agriculture by exposure to animal sources of infection resulted in a very few cases of tuberculosis. Exposure of test animals in cages in rooms with human beings in Germany, France and this country has almost invariably resulted in the development of the disease in some of the animals, but unfortunately in no

instance could contact infection be absolutely excluded except in an experiment made in a house in Providence occupied by a careless consumptive. Here two sets of guinea pigs were exposed in cages, one set fed by the patient and the other excluded from any possible form of contact. Most of the animals in both sets developed the disease. The animals in the locked cage covered with wire gauze were, I believe, infected by mouth spray, as the patient often held his face right in front of the box and talked to the animals. There is, then, a good deal of evidence to show that tuberculosis may be, oftentimes, an air-borne disease. This evidence is certainly far more convincing than for any other disease except perhaps anthrax. If, as seems probable, the air may serve as a vehicle for the carriage of tubercle bacilli it is probably because in this disease more germs are discharged indoors in places where they are likely to get into the air than is the case in other diseases, and also because the bacilli have a fairly high resisting power.

On theoretical grounds anthrax, or woolsorters' disease, ought to be more easily air-borne than any other. The spores resist drying for many years, their virulence is high and they are, at times, found in large numbers in materials handled by workmen in such a way as to give rise to much dust. Workers in wool and hair coming from anthrax-infected regions have always been much subject to this disease. Formerly, when no precaution was taken to prevent the breathing of dust, the disease was very much more prevalent than it is now. During nine months in 1882, 32 cases occurred in the woollen industry in Bradford, England, of which 23 were of the pneumonic type and presumably due to the inhalation of dust. Last year there were 17 cases and of these only three were of the internal type, so that it appears that with increased freedom from dust the latter type of the disease has shown many times the decrease shown by the external type. Careful observers in England and Switzerland have noted the occurrence of anthrax in animals grazing on pastures on which dust from wool-sorting or hair-cleansing rooms was blown.

After this review, a summary scarcely seems necessary, or even a formal conclusion. We have seen that a number of important diseases, formerly considered exclusively air-borne, have been shown never to be such. There is little evidence that among the diseases which commonly occupy our attention in this part of the world, aërial transmission is a factor of importance. In most it is, under ordinary conditions of home and hospital, a negligible factor. For tuberculosis alone is there evidence that air-borne infection is a factor of moment, but the last word has not been said as to the etiology of this disease. We may be sure that the sewer-gas bogie is laid, the notion that dust is a dangerous vehicle of every-day infection is unsupported, and that mouth spray is usually effective only at short distances.

THE ORIGIN AND EVOLUTION OF THE NERVOUS SYSTEM*

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TO the ancients what we designate as personality was a more or less general attribute of the human body rather than an aggregate of functions having a strictly nervous source. In fact, Aristotle, who was such an accurate observer and profound thinker in so many fields of biology, denied positively that the brain was in any direct way concerned with sensation and declared the heart to be the sensorium commune for the whole body. To Galen is ascribed the belief that the brain was the seat of the rational soul, the heart the location of courage and fear, and the liver that of love. This distribution of the element of personality over the physical body finds its expression in the common speech of to-day, particularly in relation to the heart, which is widely accepted by the popular mind as the source of the more tender emotions. It was chiefly through the anatomists and physiologists of the early Renaissance that the modern movement, which has tended to limit personality to the nervous system, was seriously begun, a movement which, with the increase of knowledge, has gained support to such an extent that it can now be maintained beyond any reasonable doubt. Human personality is in no true sense the outcome of the non-nervous organs, such as the digestive or the circulatory organs, but is the direct product of the nervous system. This system, to be sure, is embedded among the other organs of the body and the environment thus provided influences profoundly its condition and action, but what is meant by individual personality, acuteness or dulness of sense, quickness or slowness of action, tempera-

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mental traits, such as a gloomy or bright disposition, incapacity, shiftlessness, honesty, thriftiness or sweetness, are all, strictly speaking, functions of the nervous organs. Although only the higher animals can be said to possess personality in this sense, traces of it occur in the lower forms and its evolution is indissolubly connected with that of the nervous system. It is the object of this paper to trace in broad outlines the development of those organs which in the higher animals come to be the seat of personality.

The nervous organs of the higher animals, including man, consist of enormously intricate systems of interwoven nerve-cells or neurones whose unique character was first fully grasped some twenty years ago by Waldeyer. These neurones, like other cells, possess a nucleated cell-body, the ganglion-cell of the older neurologists, from which extremely attenuated processes, the nerve-fibres, reach out to the most distant parts of the animal. These processes are the most characteristic parts of the neurone. Extending as they do in the largest animals for some metres from their cell-bodies, they afford an example of a cell process such as is seen in no other histological unit. Not only are the nerve-cells or neurones thus highly specialized in their structure, but they also exhibit profound physiological differentiation. Thus among the primary sensory neurones each one is connected, as a rule, with a particular portion of the animal for which no other neurone is responsible, and among the motor neurones each one controls a group of muscle-fibres not called into action by any other neurone. Hence functional specialization among these elements has come to be so extreme that the nervous system may be described as one in which differentiation has reached to its very cells, a condition that is shown in no other elements of the body except possibly in the reproductive cells.

Notwithstanding the high degree of differentiation exhibited by the neurones of the higher animals, these elements may be easily grouped into relatively few classes distinguishable through their connections. These classes are three in number: first, the afferent, or as they are commonly called, the sensory neurones extending in general from the surface of the animal to the cen-

tral organs and transmitting sensory impulses; secondly, the efferent neurones connecting the central organs with the muscles, glands, etc., and transmitting efferent impulses; and finally, what may be called the association neurones, to extend to the whole nervous system a term used by Flechsig for elements in a limited part of the brain, or those neurones which lie entirely within the central organ and connect one part of this organ with another. Although the nervous organs of the higher animals are composed of an abundance of all three classes of neurones, the association neurones in all probability far outnumber those of the other two classes and constitute the chief mass of these organs.

Almost all nervous operations in the higher animals involve all three classes of neurones. The typical nervous reaction of these animals consists of a sensory stimulation followed by a motor response. This operation has been called a reflex, to use that term in its widest sense, that is, irrespective of the association of the action with voluntary or conscious operations. Such a reflex takes place over an arc of neurones, the sensory members transmitting to the association elements, and these in turn to the motor elements, but in describing the reflex its parts are not conveniently dealt with from the standpoint of the neurone. The reflex, as ordinarily understood, begins with the activity of a sense-organ or receptor, from which a sensory impulse passes to the central nervous system or adjustor, whence the nervous disturbance makes its way to the third element or effector, usually a muscle. The sense-organs or receptors are, for the most part, the distal ends of sensory neurones. The central organs or adjustors include the proximal ends of these elements, all the association neurones, and the proximal ends of the efferent neurones. The effectors are not neurones at all, but muscle-fibres, gland cells or other types of cells under the control of nerves. Thus the ordinary reflex may be said to involve in sequence the activity of a receptor, adjustor and an effector, to use modern terminology, and these three elements are recognizable in every complete reflex arc.

Our own reflexes are sometimes associated with consciousness

and sometimes not. When we pass from a region of dim light to one of bright light the pupils of our eyes contract without our being conscious of the fact. In a similar way, when food is introduced into the digestive tract, a whole succession of reflex movements is called forth without any direct relation to our consciousness. On the other hand, if we burn a finger, it is usually withdrawn with full recognition of the sensation and the response. Thus a reflex may or may not be association with a conscious state.

From this standpoint, what is the condition in the lower animals? Have they nervous systems composed of neurones and exhibiting reflexes which in some instances are associated with consciousness, and in others not? In other words, what have been the steps by which has developed that mechanism which serves us at once as the means of our simplest reflexes and the material basis for our intellectual life?

As an example of the lower animals whose nervous activities are worthy of consideration we may take the earth-worm. This animal has at its anterior end a small brain from which a ventral ganglionic chain extends posteriorly through the rest of its body. It possesses sensory neurones which extend from the skin into the central nervous organ and motor neurones reaching from the central organs to the muscles. The central organ itself contains association neurones. Thus the three classes of nervous cells which occur in man are also represented in the earth-worm but with this difference. The association neurones, which in man are relatively very numerous, are in the earth-worm comparatively few. Otherwise the essential composition of the nervous organs in these two forms has much in common.

Not only is the nervous system of the earth-worm composed of elements essentially similar to those of the higher animals, but it exhibits similar functional relations. The earth-worm responds to a large range of stimuli by appropriate and characteristic reactions, and its movements justify the conclusion that its reflex arcs, like those of the higher animals, involve receptors, an adjustor, and effectors.

Whether certain of the reflexes of the earth-worm are asso-

ciated with consciousness or not is a question that cannot be answered definitely, since no absolute criterion for consciousness in any organism other than one's self can be given. Earthworms, however, apparently possess some capacity to profit by experience. Within the past year Yerkes has reported on the training of an earth-worm which in a surprisingly short time acquired the habit of escaping successfully from a very simple maze. These results, should they prove true for other individuals, suggest a certain degree of consciousness in these creatures as a basis of their ability to learn. It is, therefore, not impossible that certain of the reflexes of earth-worms may be associated with conscious states, even though these states may be of a very low order.

But, though the reflexes of the lower animals show some features that suggest consciousness, it is not probable that this state is anything like as characteristic of these simple forms as of the more complex ones. Certainly some of the performances of these more primitive beings have every mark of the unconscious reflexes of our own bodies. Thus bees that have been artificially hatched and have never seen the colony at work make as perfect comb as though they had learned the art by having been co-workers in an established hive. Such bees, moreover, will not only build comb such as they themselves were hatched from, but will shape a queen cell, a form with which they have had absolutely not the least acquaintance in the past. Thus the very complex operation of comb-building in the bee resembles our own unconscious inborn reflexes, such as the constriction of the pupil and the movements of the digestive tube, rather than our voluntary operations, and this is probably true of many of the activities of the lower animals. In fact, it seems fair to conclude that, though such animals as the insects, crabs, and even the worms possess a nervous system composed of elements similar to those in the higher forms, their reflexes are much more mechanical and less associated with anything that can be called a conscious state than are those of the higher forms. In other words, these lower animals are more in the nature of reflex machines than are the higher forms, though

they are not, as some investigators would have us believe, exclusively so.

But if the nervous system in many of the lower animals is composed of elements similar to those in the higher forms, and exhibits activities not unlike our own, are there not still more primitive animals in which this system shows a real reduction and exhibits a condition which marks the actual beginnings of nervous organization? Such primitive forms have long been supposed to exist among the cœlenterates and are well represented by the sea-anemones.

Sea-anemones are sack-like animals with a single opening leading into the digestive cavity and serving both as mouth and anus. This opening is usually surrounded by a cluster of tentacles. The living body of the sea-anemone consists of the thin membranous wall that separates the digestive cavity from the outer sea-water, and that is drawn out in processes to form the hollow tentacles. In no part of its structure is the sea-anemone massive, as is the case in most higher forms, where the muscles, skeleton and so forth usually give rise to a considerable thickness of tissue; in fact, the animal exhibits no well-defined organs except the digestive organs, and may be described as a membranous digestive sac.

Although the body of the sea-anemone is really nothing more than membranous walls, these walls have long been known to contain both nerve and muscle. These two tissues occur over almost the whole animal. According to the Hertwigs, the nervous tissue is more abundant in the neighborhood of the mouth than elsewhere, and this region has been regarded by some investigators as a central nervous organ. But the studies of Jordan and others have shown conclusively that this opinion is not correct, and that the removal of this region interferes in no serious way with the reactions of the animal. Apparently each part of the sea-anemone carries with it its own neuro-muscular mechanism, a condition well illustrated by the tentacles. These organs are chiefly concerned with appropriating the food and are stimulated by the dissolved materials in the food. A tentacle when cut off from a sea-anemone and held in sea-

water can still be stimulated by food and will exhibit almost exactly the same kind of movements when thus isolated that it did when a part of the whole animal, thus demonstrating the completeness and independence of its own neuromuscular mechanism. Nervous transmission can be accomplished from almost any part of the sea-anemone to almost any other part, but, as such experiments as those with the tentacles indicate, no one part of the animal's nervous organization seems to be more important than any other part. In other words, the nervous system in the sea-anemone is diffuse rather than centralized.

When the minute organization of the nervous system of these animals is studied, it is found to consist of a vast number of sensory neurones which connect the surface of the animal with the underlying muscles and which form there what appears to be an intricate nervous network. This nervous mechanism is concerned primarily with the reception of stimuli and the immediate excitation of the muscles. The nervous mechanism is a receptor mechanism that acts as a trigger for setting off the muscle. The whole neuromuscular apparatus seems to be made up of those two elements which in the higher animals were designated receptors and effectors and without the intervention of an adjustor or central nervous organ. Viewed from the standpoint of development, this condition points indubitably to the conclusion that the central nervous organs were evolved only after the appearance of sense-organs and muscle, and that such animals as the sea-anemone may well be taken to represent this step in the evolution of the nervous system. This general view of the origin of the central nervous organs was advanced as early as 1886 by Kleinenberg and was reaffirmed ten years later by Rakowitza.

The evolution of nerve and muscle, so far as this problem can be attacked in such lowly forms as the sea-anemone and other cœlenterates, is a question about which there has been much difference of opinion. As early as 1872 Kleinenberg showed that in the fresh-water cœlenterate, *Hydra*, there were certain peculiar T-shaped cells that he called neuromuscular cells and that he believed to represent both nerve and muscle.

In these cells the arm of the T reached the surface of the animal and was thought by Kleinenberg to act as a nervous receptor; the cross-piece being contractile was known to be muscle. Kleinenberg assumed that the division of such cells and the differentiation of their parts were the processes which gave rise to the nervous and muscular tissues of the higher animal. In 1879 the Hertwigs in their account of the structure of sea-anemones showed that the so-called neuromuscular cells of Kleinenberg were in reality simply epithelio-muscle cells and were without nervous significance. These investigators, in opposition to Kleinenberg, advanced the view that nerve and muscle, though simultaneously differentiated, were derived from different groups of cells. According to both Kleinenberg and the Hertwigs nerve and muscle were simultaneously evolved, but Kleinenberg maintained that these tissues came from a single form of cell, the Hertwigs that they arose from separate kinds of cells.

My own studies on the origin of nerve and muscle have led to rather different conclusions from those summarized in the last paragraph. In studying the reactions of one of our common sponges, *Stylotella,* I was impressed with the extreme slowness with which the animal responded to a stimulus. The oscula of this sponge can be made to close by the application of several kinds of stimuli. The closure of these openings is accomplished by the contraction of the ring of muscular tissue surrounding them. This response occurs some minutes after the stimulus has been applied, a condition in strong contrast with the quick reactions of such animals as sea-anemones. These forms respond to most stimuli within a second or so, the sponges only within minutes. Moreover, in sponges transmission from the place where the stimulus is applied to the responding muscle is possible only over very short distances and is carried on at a very slow rate. Transmission in *Stylotella* resembles very closely the kind of transmission seen in ciliated epithelium. The successive beat of the cilia is dependent upon an impulse which progresses from cell to cell in the epithelium at a relatively slow rate and is neither purely mechanical nor nervous in its method of

propagation. It probably represents a primitive form of protoplasmic transmission, a forerunner of the true nervous impulse, and as such gives us some insight into the nature of the non-nervous transmission in sponges. The results of my studies on *Stylotella* support the conclusions of most biologists who have worked upon sponges, that these animals probably possess no true nervous tissue. Their muscles, in my opinion, are brought into action almost entirely by the direct effect of the stimulus rather than through nerves, and this accounts, I believe, for their very slow response to external disturbances. It is possible that in certain sponges some form of nervous tissue may be demonstrated eventually, or that such organs as those described by von Lendenfeldt as synocils may be shown to have a sensory significance, but such cases, if they do occur, will probably remain exceptional, for as a whole sponges seem to be a group of animals almost if not quite devoid of true nervous tissue. Granting this conclusion, it must be evident that the condition in sponges throws a very important light on the question of the origin of nerve and muscle. Their state suggests at once that nerve and muscle have not been differentiated simultaneously, as maintained by Kleinenberg, the Hertwigs, and others, but that muscle preceded nerve in its evolution and that sponges represent animals with effectors but without differentiated receptors. If then it may be claimed that phylogenetically the sense-organ preceded the central nervous organ, it may also be maintained that muscles preceded sense-organs. Thus the three elements of the reflex arc of the higher animals were probably evolved separately and in the order, effector, receptor, adjustor.

If muscle originated before nerve and was brought into action at first by direct stimulation, it is natural to expect that examples of this form of response might still be found among the higher animals. And such seems to be the case. Thus the sphincter of the iris in the lower vertebrates, though well known to be under the influence of nerves, was shown by Steinach some time ago to be directly stimulated by light, a condition which, judging from the more recent work of Hertel, probably applies

even to the human eye. This muscle then exhibits a certain capacity for normal direct stimulation. Another example of the same kind is seen in the embryonic vertebrate heart. Though the beat of the adult heart may be a matter of controversy from the standpoint of the myogenic and neurogenic theories, there can be no doubt that the muscle of the embryonic heart beats, as shown by His, before it has become invaded by nerves. And this view is supported by Burrows' recent discovery that the isolated cells of the heart-muscle will contract rhythmically under conditions where not the least vestige of a nerve can influence them. Thus the embryonic heart-muscle and the sphincter of the iris are muscles whose activity may be normally called forth by direct stimulation, a condition which reproduces, so far as independence is concerned, the state met with in the muscles of the sponges. These examples then show that even in the higher animals certain muscles respond normally to direct stimulation and thus exhibit a form of activity which is believed to be generally characteristic of sponges.

In my opinion the simultaneous origin of nerve and muscle can no longer be maintained. Muscle arose first and the simple effectors thus produced were the first element of the neuromuscular mechanism. These effectors were directly stimulated and consequently slow in action. They afforded centres around which nervous tissue first differentiated in the form of sense-organs or receptors whose function it was to serve as triggers to initiate muscle action quickly. As these receptors became more highly developed, a third element, the central nervous organ, arose from the nervous elements between the receptor and the effector. This organ, the adjustor, served as a means of conducting and modifying the sensory impulses on their way from the receptor to the effector, and ultimately it also served as a storehouse for the nervous experience of the individual and as the seat of its intellectual life. It is interesting to observe that this view of the origin of the nervous system is in accord with the philosophical speculations of Bergson, according to whom the nervous system has been evolved pri-

marily as an organ for animal response and only secondarily as one concerned with intellectual activities.

But if we picture the nervous system as having arisen as an appendage to the musculature and as having grown in complication as the musculature became differentiated, we are still far from an adequate view of even the more obvious aspects of its evolution. The nervous system controls many more kinds of effectors than muscle and its sensory elements are vastly more complex than is implied in the preceding sketch. To gain a more comprehensive view of the evolution of these organs, it is necessary to consider a subsidiary but important process, the appropriation of effectors and receptors.

The nervous system of many of the higher animals not only acts upon the musculature; it may also control electric organs, luminous organs, chromatophores, glands, etc. Not all such organs are under the influence of the nervous system, but it is not difficult to find for each group of effectors animals in which the given type of organs is under the influence of nerves. The electric organs and the chromatophores of fishes are of this kind as well as the salivary glands of the mammals and the luminous organs of the brittle stars.

How has the nervous system gained control over these organs? Except the electric organs, which are probably modified muscle, all these organs have arisen in my opinion as independent effectors. Most of them can be identified as such in one group of animals or another. Thus among the glands the pancreas in the higher vertebrates has been shown to be in its action essentially non-nervous. Such highly differentiated, but independent effectors have, I believe, been appropriated from time to time by the nervous system in that during ontogenesis certain motor fibres, instead of becoming attached to their appropriate muscles, have wandered to new effectors which have been sufficiently responsive to their stimuli to give a basis for a permanent attachment. Thus the nervous system, once established around muscles, has widened its influence in that it has appropriated other types of independent effectors, which upon application were found to be responsive to its stimulus.

But the differentiated nervous system has not only extended itself on the side of its effectors, it has probably also made receptor appropriations. This is well illustrated by several groups of related sense-organs such as the organs of touch and hearing in the vertebrates or those of the chemical senses in the same animals. The latter may serve as an example.

The chemical sense-organs in vertebrates include not only those of smell and of taste, but also the organs of the common chemical sense such as are concerned with the chemical irritability of the skin of the frog or of the exposed or semi-exposed mucous surfaces of man. All these chemical receptors are stimulated by solutions. In taste the stimuli are the dissolved materials in the food; in smell they are the solutions formed on the moist olfactory surface from the materials wafted in the air to the nose.

The neurones concerned with the reception of these stimuli exhibit interesting relations. The olfactory neurones, as is well known, have their cell bodies in the olfactory epithelium, whence their neurits extend into the central olfactory apparatus. They reproduce in a most striking way the type of primary sensory neurone common to the invertebrates, and in this respect they represent the most primitive type of sensory neurone in the body of vertebrates. The neurones concerned with the common chemical sense are like those of the olfactory sense except that their cell-bodies have migrated centrally and constitute a part of one of the cerebrospinal ganglia. As a result the distal ends of these neurones are represented as free-nerve terminations in the epithelium of the moist parts of the vertebrate skin. The gustatory neurones reproduce almost exactly the condition of those of the common chemical sense, except that their distal free terminations are around taste-buds instead of being in an ordinary epithelium.

The conditions shown by these three types of receptor mechanisms suggest at once a genetic connection. The olfactory type is undoubtedly the most primitive, and stimulation in this instance is initiated by the chemical action of the superimposed solution on the hairs of the olfactory cells. The neurone

for the common chemical sense has probably been derived from one of the olfactory type by a proximal migration of its cell-body. The stimulation of its free-nerve terminals may be conceived to take place, as Botezat has recently pointed out, through the secretory activity of the surrounding epithelial cells as a result of their contact with the stimulating solution, rather than from the direct action of this solution on the nerve-endings themselves. From this standpoint the epithelium comes to be an essential element in the stimulation of the neurone and affords, so to speak, a favorable sensory environment for the real nerve-endings. Finally, the gustatory neurones may be said to have appropriated certain of these epithelial cells which have become differentiated into taste-buds and whose activity, probably secretory in character, to follow Botezat, is called forth by the superimposed solution and is essential to the stimulation of the nerve-endings.

Thus in the evolution of the chemical sense-organs of vertebrates certain integumentary cells originally quite independent of the receptors came to be involved with these and were eventually appropriated by them as essential parts of the gustatory apparatus. This process of appropriation is not unlike that seen among the effectors and represents one of the important steps by which the nervous system in the course of its evolution has added to its complexity. Although the nervous system probably arose in a scattered way at spots where the primitive multicellular animal had developed muscle, it became unified through the need for common transmission tracts, and, by increasing its own elements as well as by appropriating additional effectors and receptors, it has impressed upon the higher animals, including ourselves, a unity so profound that it includes everything that we mean by personality.

PNEUMOCOCCUS INFECTION AND LOBAR PNEUMONIA*

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PNEUMONIA, in many respects, certainly as a cause of death, is the most important infectious disease with which we have to deal. The symptomatic treatment is difficult and of doubtful utility; there is no well-established form of specific therapy. Nevertheless, up to within a very recent time, the investigation of the real nature of the process has been slight and fragmentary.

While the association of certain kinds of bacteria with this disease has been well established, much obscurity exists with regard to the mode of infection, the relation of the bacteria to the lesions and symptoms, the nature of recovery and, above all, with regard to the possibility of prevention or of cure. It has been in the hope of helping to shed some light on these problems that my associates and I have been making some clinical and experimental studies. It will be impossible to review in this paper all the work that has been done by others, and I shall have to content myself with presenting certain points of view which have been suggested mainly by our own work at the Rockefeller Institute.

Acute lobar pneumonia seems the best characterized of the acute lung affections. It has such a clear-cut clinical course that it is now generally considered a distinct clinical entity, and no more to be regarded simply as an infection of the lung than typhoid fever is to be considered an infection of the intestine. This, however, is a clinical point of view and it is possible that the same kinds of reaction occur in other localized pneumococ-

* Delivered December 13, 1913.

cus infections as are present when the main seat of infection is the lung.

In a very large percentage of patients suffering from inflammation of the lung of the lobar type, pneumococci are present in the lesion. In isolated instances, other organisms, as *Bacillus influenzæ* or *B. pneumoniæ*, are found in pure culture. It has not been our purpose to consider these latter cases, but our attention has been given entirely to the group of cases in which *Diplococcus pneumoniæ* is present and is apparently the etiological agent. As is well known, diplococci, which at present cannot be differentiated from the pneumococcus, may be present in pneumonia of the lobular type (in which the clinical course is quite distinct from that in lobar pneumonia); they may also be present in other purely localized lesions in the body, entirely unassociated with any affection of the lung, and they may even be the organisms concerned in certain cases of septicæmia in man without any local lesions whatever. Moreover, organisms with identical characteristics, so far as yet determined, are found with so great frequency living on the mucous membranes of the mouth and throat of perfectly healthy individuals that they may be considered normal inhabitants of the mouth and throat cavities. In the face of such facts as these, how can it be maintained that *Diplococcus pneumoniæ* is the primary cause of such a well-characterized acute infectious disease as acute lobar pneumonia? In view of the present general consensus of opinion that this theory is true, one is indeed rash even to suggest the possibility that there may be another agent concerned. On the other hand, it is important that such a possibility should not be overlooked. Even though it should be shown, however, that pneumococci do not play the primary etiologic rôle in the natural infection, their association with the lesion and their frequent invasion of the blood render it evident that they play an important part in the process and probably the most important part in the outcome, just as do streptococci in certain diseases, such as smallpox and scarlet fever, of which it is generally believed that the natural infection is due to specific etiologic agents.

Up to within a relatively short time, the most important link in the chain of evidence that pneumococci cause pneumonia, namely the reproduction of the disease in animals, was lacking. Most important studies dealing with the experimental production of acute lobar pneumonia were published in 1904 by Wadsworth.[1] By carefully balancing the general resistance of the animal with the virulence of the race of pneumococci employed and by injecting the organisms intratracheally, he was able, in a series of rabbits, to induce a diffuse exudative pneumonia like the acute lobar pneumonia seen in man.

More recently Lamar and Meltzer,[2] and Wollstein and Meltzer[3] have succeeded in regularly producing a diffuse pneumonia of the lobar type in dogs, by injecting from 10 to 15 c.c. of the fluid culture directly into one bronchus through a rubber tube passed through the trachea, following the injection of the fluid by air blown through the tube, so as to force the infectious material into the finer ramifications of the bronchi. The pneumonia produced in dogs runs a more rapid course, resolution occurs earlier—in three or four days—and the mortality is much less than in pneumonia in man.

Using a similar technic, these investigators have produced diffuse lesions in the lungs of dogs with other micro-organisms. When streptococci are injected, the lesions tend to resemble more closely those seen in bronchopneumonia in man.[4] The observers lay stress on the greater tendency in this case to a leucocytic infiltration of the lung framework, and to a much-lessened formation of fibrin. These differences between the pneumonia produced by the injection of streptococci and that following the injection of pneumococci they ascribe to inherent differences in the nature of the micro-organisms concerned, and not to relative differences in virulence. By a similar method of intratracheal injection, Winternitz and Hirschfelder[5] have succeeded in producing pneumonia of a lobar type in rabbits. In these experiments also, large amounts of the culture material (4 or 5 c.c.) were injected.

From experiments which I have carried on, using the same method, it is evident that successful results in rabbits depend

somewhat on the race of organisms employed. If the organisms have very slight virulence, the animals may recover without lung lesions; if they are too virulent, a septicæmia quickly results and at necropsy only congestion and œdema of the lungs are present.

As Wadsworth showed, the lung consolidation is probably a manifestation of the resistance of the animal to the spread of the infection. The occurrence of the diffuse lung lesion is undoubtedly dependent on the same factors which are concerned in the differences in local reaction to the injection of virulent pneumococci in different races of animals. As is well known, mice and rabbits are very susceptible to pneumococcus infection, however induced, guinea pigs and dogs less and man possibly still less. The result of a subcutaneous injection of virulent pneumococci into a rabbit differs markedly from that seen when a similar injection is made into a guinea pig. In the former there is very little local reaction; a rapid general invasion of the organisms takes place, and the animal dies quickly from a marked septicæmia. In the latter a marked infiltration with much fibrin formation and a slowly progressive invasion of the subcutaneous tissues occur, while there is little or no general infection. That the time element plays a rôle in the formation of fibrin is well seen in the peritonitis induced in such susceptible animals as the mouse and rabbit. If an intraperitoneal injection is made of virulent pneumococci, death occurs within twenty-four hours, and in the peritoneum there is seen only a marked congestion, possibly hemorrhages, a serous exudation and usually no fibrin. If, however, the culture is less virulent and the animal lives forty-eight hours or more, there is usually considerable fibrin over the liver, and flakes of fibrin are seen throughout the cavity. The amount of fibrin increases in ratio with the length of time during which the animal is able to resist the infection.

The experiments of Wollstein and Meltzer,[4] however, tending to show that the peculiar property of stimulating the production of fibrin is possessed to a greater degree by pneumococci, whatever their virulence, than by streptococci, is of

very great significance. It is difficult to understand, however, just why this property should be a factor in the production of pneumonia of a lobar rather than of a lobular type. That this peculiar property should not be the only one concerned is made evident from the fact that the pneumococcus is the organism most frequently concerned in lobular pneumonia in children. Dochez[6] has shown that, during the acute stages of lobar pneumonia, there is an increase of fibrinogen in the blood. Nevertheless, the coagulation time of the blood is delayed, owing probably to an increase in antithrombin as well. We know of no observations which show whether or not so great an amount of fibrinogen exists in the blood in prolonged streptococcus infections in man as is present in pneumonia.

As the virulence of the race employed is of importance as regards the production of the local reaction, so also is the number of micro-organisms injected, as Kline and Winternitz[7] have shown. If the number of organisms is too small, no pneumonia results. It is well known that even in very susceptible animals a considerable number of virulent organisms is usually necessary to produce infection. Little attention has been given to the question why, when a considerable number of organisms are injected together, multiplication occurs and infection results, whereas if only a few organisms be injected, they are unable to multiply. Is it because in a culture of organisms certain ones are more resistant than others to the harmful influences, or is it simply accidental that, when a large number of organisms are injected, a few have a possible chance to escape?

Gillespie[8] has carried on some experiments with pneumococci which have a bearing on this problem. We, as well as others, have long recognized that in starting a culture of pneumococci in a large amount of bouillon, a litre for instance, a much larger inoculation is necessary in order to obtain growth than if the inoculation be made into 10 c.c., or if the culture be made on a solid medium. In the latter instance, one organism will usually produce a colony. By making the inoculations on filter-paper kept constantly wet by bouillon, it was shown that growth would occur with the inoculation of as small numbers

of organisms as are required on agar, and with much smaller numbers than are required to inoculate the bouillon. The differences in growth, therefore, are not dependent on differences in composition of the medium, and further experiments have shown that they are not due to differences in oxygen supply. It seems probable that for growth to occur, the bacterium must produce changes in the medium immediately surrounding it, and that when the opportunities for diffusion are great, such local changes cannot be kept sufficiently constant unless there be a considerable number of organisms in proximity. If this be the true explanation, it may have an important bearing on infection, not only with pneumococcus but with other micro-organisms as well. The presence of mucus in the smaller bronchi, for instance, might in this way favor the multiplication of micro-organisms and so favor infection. That very large numbers of bacteria are inoculated in the experiments of Meltzer and Winternitz probably explains to some degree why their experiments have been successful, where others have failed. A second factor in the success of this technic probably lies in the fact that considerable amounts of fluid are injected and this is blown into the terminal bronchioles. Meltzer has made the interesting suggestion that by this process the bronchioles are occluded and that in this way closed cavities are formed. It is generally recognized that as long as bacteria grow on exposed surfaces, they do no harm. It is only when the growth occurs in confined spaces that harmful results supervene.

While these experiments on the production of pneumonia in animals are of great value in showing that lesions resembling acute lobar pneumonia in man may be caused by pneumococci, they do not directly offer an explanation of the natural infection in man. It is hardly likely that in man an overwhelming infection ever occurs with numbers of bacteria so large as those used in the experiments in dogs. The usual recourse in this dilemma is to assume that the organisms concerned in natural infection have an increased virulence, or that the resistance of the host is lowered. By virulence in micro-organisms is usually meant adaptation to growth in the tissues of the host.

Since in pneumonia the organisms are cultivated from the lungs and at times from the blood, we know that they have virulence for man. We have no way of determining, however, whether or not all pneumococci growing in the mouths of healthy individuals are also virulent for man. The attempts to demonstrate conclusively that pneumococci isolated from cases of pneumonia in man are regularly of increased virulence for animals have not been successful. We have found that most of the pneumococci isolated from the blood of pneumonia patients have relatively high virulence for susceptible animals, yet while certain of these cultures, when freshly isolated, are of such virulence that 0.000001 c.c. of a bouillon culture will kill a mouse, we have also obtained cultures that required 0.5 c.c. to kill. The virulence of the race, therefore, does not seem to be the only deciding factor in the question why infection occurs, even though, as will be shown, it may be of considerable importance as regards the final outcome. It must be stated, however, that it is not certain that virulence for animals is identical with virulence for man. Usually high virulence of a given race of pneumococci for one susceptible animal, as the rabbit, indicates high virulence for another, as the mouse. Ungermann, however, has described a typical pneumococcus having high virulence for rabbits, but its virulence for mice, which was originally present, was lost. We have studied a race of pneumococci originally very virulent for mice, which, after passage through guinea pigs, increased its virulence for these animals, but became almost avirulent for mice and rabbits.

Against the view that pneumonia arises when organisms of increased virulence reach the lung is the fact that pneumonia rarely occurs in epidemics and is very slightly contagious. There are now a number of epidemic outbreaks reported, but it must be admitted that these are of rare occurrence, and all hospital experience is against contagion as a great factor in the spread of the disease. On the other hand, the fact that in certain times and places pneumonia occurs with greatly increased severity and frequency suggests that at these times the pneumococci concerned may have acquired increased virulence.

When the French attempted to build the Panama Canal, the incidence and mortality of pneumonia interfered as seriously with the work as did the occurrence of malaria and yellow fever. Even during the first years of the American occupation of Panama, the mortality from pneumonia was enormous. For its decrease there seems no adequate explanation. In South Africa, deaths from lobar pneumonia among the coolies constitute a serious menace to the continued working of the mines.

Little is known concerning diminished general resistance on the part of man to pneumococcus infection and its importance in natural infection of this disease. Clinical studies have quite conclusively demonstrated that the habitual use of alcohol increases susceptibility to infection or, at any rate, renders the subject less resistant when infection has once occurred. That exposure to cold, or especially sudden changes in temperature and chilling, play a part in infection can hardly be doubted, and there is some experimental evidence to show that animals suddenly chilled are more susceptible to infection with pneumococci than others.

The view that local changes in the lung are of importance for the occurrence of infection is interesting and suggestive. In most cases of lobar pneumonia the primary seat of infection is probably in the lung. Various writers have attempted to show that infection occurs through the blood-stream, but the evidence is not conclusive. Other localized pneumococcus infections in internal organs or cavities usually occur by extension, though this is not always demonstrable. The possibility that infection through the blood may occur in certain instances cannot be excluded. Pneumonia, as a part of a general infection, however, is generally of a lobular type.

Much has been said lately about the adaptation of certain organisms for certain tissues. In many cases, however, this adaptation is more apparent than real, and the mode and degree of infection play the larger rôle in localization.

In about 50 per cent. of the cases of pneumonia, a history of preceding coryza and cough may be obtained. In these cases it is possible that there occurs a downward infection along the

mucous membrane of the bronchi. The extension of the lesion through the lung from one lobe to another apparently takes place through the bronchi, as the study of large sections through lobes with beginning involvement shows. In the remaining half of the cases, however, the onset is sharp and sudden without history of bronchial involvement. Even in these cases some local change probably precedes the real infection. It is well known that thoracic trauma is frequently followed by pneumonia. The idea of Meltzer that infection may be facilitated by closure of the smaller bronchioles is most suggestive. It is possible that cold or chilling may stimulate the mucous glands so that the increase of mucus may produce favorable conditions for the growth of pneumococci, which are so frequently present in the upper respiratory tract.

That the lung is the chief seat of the disease, then, is probably due to the fact that infection occurs here, and that a local lesion results and not a general infection (at least not until late in the disease) is probably due to the fact that man is highly resistant to infection with pneumococci and that the anatomical conditions here permit of an extensive inflammatory reaction which opposes the spread of the infection.

But why does infection occur at all? Why does a person contract pneumonia? There is still considerable obscurity in regard to this phase of infection, not only in pneumonia, but also in many other infectious diseases of which the etiology is well known. The obscurity and difference of opinion in regard to tuberculosis are well known. Even our views with regard to infection in diphtheria have been disturbed by new observations. We all thought the transference of the infectious agent from the infected to the healthy throat was all that was necessary for infection in this disease. The observations of Moss, Guthrie and Gelien,[9] however, that in Baltimore there are four times as many carriers of virulent diphtheria bacilli as there are cases of the disease, are most disturbing. So far as can be determined, the bacilli from carriers differ in no way from the bacilli from patients with the disease. Moreover, the incidence of the disease seems to bear no relation to these carriers. The problem of

mode of infection is thus in a minor degree analogous to that in pneumonia, in which practically the entire population represent carriers of infection.

To explain the nature of infection we may say: First, there is a possibility that in pneumonia, as in diphtheria, the organisms causing infection differ inherently from those in normal throats, especially as regards adaptation or virulence for men. Definite evidence in favor of this would be most important, but at present there is none. Second, it is possible that the general resistance of patients to pneumococci is lowered, so that organisms, formerly living as harmless parasites, now invade the tissues and induce reaction. For this also we have no definite evidence. Third, the study of artificial infection in animals, as well as the course of the disease in man, suggests most strongly that local changes in the respiratory tract may precede the infection with pneumococci. Whether these are due to a primary infectious agent or to changes in the tissues due to other factors cannot be decided definitely at present. Finally, it is possible that infection depends on a combination of factors, virulence of organisms and general and local resistance each playing a part. Further knowledge along these lines is absolutely essential for prevention of this disease. To formulate rules or regulations for prevention at present seems useless, except as an experiment.

THE NATURE OF THE INTOXICATION

Whatever be the mode of onset in pneumonia, the production of the local changes in the lungs, as well as the general systemic manifestations of the disease, seems to be in some way related to the growth of pneumococci in the body. When micro-organisms grow within other multicellular organisms acting as host, the effects on the host are of two kinds: First, there is a local reaction, in which the bacteria are present in considerable numbers, as at the point of infection. Here are induced the changes spoken of as inflammation. In addition to this, however, there is practically always a reaction throughout the entire body, even when the local reaction is very mild and

evanescent. These general reactions are evidenced, not only by fever and nervous disturbances on the part of the host, but even in their absence by such effects as changes in the blood, especially the leucocytes, which indicate certain effects on the blood-forming tissues.

In most cases, the exact manner in which micro-organisms stimulate the tissues in which they are growing to a reaction which is called inflammatory, is still obscure. Since, however, identical reactions may be produced with non-living chemical substances, it is generally assumed that in the case of bacteria as well, non-living chemical substances are formed as a result of the bacterial growth, which substances are in themselves harmful. Whatever may be the exact relationship of the organisms to the local lesion, it is necessary to assume that the general manifestations of infection, and especially the effects on tissues far distant from the local lesion, are the result of soluble toxic substances which circulate in the blood or lymph. Since general manifestations similar to those in pneumonia are seen in other pneumococcus infections and even in general infections in animals without local lesions, it does not seem probable that these effects in pneumonia are due to disturbances in respiration associated directly with the lung lesions. It is possible, of course, that in pneumonia the general manifestations and effects on distant tissues and organs are due to the action *in situ* of bacteria which have gained access to the circulation and have been carried to these distant parts.

Very numerous observations have been made on the occurrence of bacteria in the general circulation in acute lobar pneumonia. During the past years blood-cultures have been made on most of the cases of pneumonia coming under my observation, and the results have not led me to change the conclusions arrived at ten years ago from the study of a series of cases, namely, that pneumococci are usually found in the blood only in the more severe cases, and the presence of the pneumococcus in the blood is of ill omen. It is possible that in all cases of pneumonia an occasional bacterium may be carried into the circulation, but the demonstration of this is difficult. That this may

occur, however, is not of prime importance, for the occurrence of an occasional organism could hardly explain the great degree of effect in distant tissues, as manifested by the general symptoms which we call intoxication.

The attempts to discover something of the nature of this circulating poison have been attended with much difficulty. It would seem that a more accurate knowledge of the metabolic disturbances in pneumonia might give a clue as to the nature of the intoxication. A series of studies with this object in view was undertaken.

Of late years attention has been drawn to the occurrence of functional disturbances, especially in infants, due to derangements in salt metabolism. It appeared of interest to learn whether or not specific changes in inorganic metabolism may be induced by pneumococci, which could account, in part at least, for the symptoms induced. The most striking disturbance in pneumonia is known to be the retention of chlorides, which is frequently almost complete during the acute course of the disease. Retention of chlorides to a lesser degree is known to occur in other infections, but Rowntree[10] has shown that this retention does not occur in influenzal pneumonia to nearly so marked a degree as it does in pneumococcus pneumonia. Medigreceanu[11] has carried on a series of studies of pneumonia in dogs and Peabody[12] has studied the question in cases in man. Peabody has shown that there is a retention not only of chlorine, but also of sodium and calcium while there is no retention of potassium and magnesium, but may be a loss. Further studies indicate that the retained substances are not stored in any one place, but are spread diffusely throughout the tissues.

It is not believed that these changes are specific for pneumonia, for they probably occur in other infections. They are most striking in pneumonia, since the changes between the febrile and afebrile state occur with such suddenness. It is not likely that these changes in themselves are responsible for any of the symptoms of the disease, but in view of the striking effects which have been induced by Meltzer by changing the balance in the inorganic salts in the body, this possibility must

be borne in mind. We have no knowledge of the reason for these changes in pneumococcus infection.

Pneumococci are known to produce acid readily, even, as shown by Hiss in albuminous mediums, containing no demonstrable sugar. It has therefore been suggested that the symptoms in pneumonia are the manifestations of an acidosis. Hamburger[13] has attempted to explain the chlorine retention on the basis of a febrile acidosis. The studies of Peabody,[14] however, have shown that the curves of chlorine retention and of ammonia excretion, which is generally considered the best indicator of acidosis, do not necessarily run parallel. The studies of inorganic metabolism have therefore given no conclusive insight into the nature of the intoxication.

In order, if possible, to obtain some knowledge regarding this problem by another method, Peabody has made studies of the gas exchange in the blood in pneumonia. He has found that in this disease the carbon dioxide in the venous blood is quite regularly low, in spite of the disturbances in gas exchange in the lung. At the same time there occurs an increase in the ammonia nitrogen in the urine, and the curves run somewhat parallel. These changes, which are indicative of increased acid formation, nevertheless correspond to changes that have been known to occur during fever and infection due to other causes, and are no indication of specific changes occurring in this disease. The carbon dioxide content of the blood does not bear a definite relationship to the severity of the disease, except that it is lowest in the most severe cases and in the terminal stages.

On the other hand, the study of the oxygen-content of the blood has revealed some interesting changes. Studies of the peripheral venous blood showed in certain cases a diminution in the oxygen-content of the venous blood. In studying the blood in one such case, it was found that the blood would not take up a normal amount of oxygen, and this in spite of the fact that the hæmoglobin content was normal. In a careful study of such blood by Butterfield and Peabody[15] it was found that this phenomenon was due to the formation of methæmoglobin. This

change also occurs regularly in the blood of rabbits[16] infected with the pneumococcus and has no relation to the lung lesion. It also occurs when the bacteria are grown in blood-containing mediums.

Usually the change into methæmoglobin in the animal body does not go so far that the methæmoglobin can be distinguished spectroscopically. In the test-tube, however, especially when hæmoglobin in solution is added to the culture, practically all the hæmoglobin may be changed into methæmoglobin. That this reaction is not simply due to the action of acids formed by the pneumococcus is shown by the fact that for the production of methæmoglobin far more acid is required than could be present in the body, and, moreover, that it may occur in cultures or filtrates that are alkaline in reaction. It is therefore evident that this change is due to the action of a poison formed by the pneumococci.

Peabody[17] further made a study of the blood in twenty-five cases of pneumonia to determine the frequency of the occurrence of this phenomenon and the time of its appearance. Of the cases which ended in recovery, in only one was there any indication of a diminution of the oxygen-absorbing power of the hæmoglobin. In all of the ten cases ending fatally, there occurred a progressive loss in the oxygen-content of the blood and in the oxygen-combining power of the hæmoglobin, and from the previous studies it is certain that these changes are due to the formation of methæmoglobin. In nine of the ten cases the blood-cultures were positive.

That these changes play a part in the fatal termination can hardly be doubted. The terminal symptoms of the disease may be accounted for by deficient oxidation. It is not likely, however, that these changes in the blood are in themselves the only factor in accounting for the fatal result; but they represent one of the factors, and are an indication of the intoxication which is the result of the growth of pneumococci in the body.

A second effect of the pneumococcus intoxication has been demonstrated by Medigreceanu[18] by estimating the amount of oxidase in the organs of animals dying from pneumococcus sep-

ticæmia, as compared with the organs of normal rabbits. In these studies Medigreceanu employed the method of Röhman and Spitzer, which is based on the property of tissues of oxidizing a mixture of naphthol and paraphenylendiamine into phenol. By comparing the tissues of normal animals with those previously infected with pneumococci, it has been found that this oxidase is generally diminished in the latter animals. By proper controls it has been possible to show that this change is due, not to the presence of pneumococci in the tests, but to some change which results in the tissue from the infection. Another effect of the action of the toxin on tissue function is thus made evident. It is therefore probable that, in addition to the lessened supply of oxygen by the blood due to the formation of methæmoglobin, there is also a lessened power of the tissues to carry on the proper oxidation function.

Finally, in order to obtain evidence of the presence of a poison, studies were made by Medigreceanu[19] to determine whether or not there was an increased output of substances known to have the property of neutralizing poisons arising in the body. Such a substance is glycuronic acid, and it was found that during the acute stages of pneumonia in man, in almost all cases, there is a definite increase in the output of this substance.

All these studies clearly indicate the activity in pneumonia of a circulating poison; but the direct demonstration of the presence of this toxic substance in the animal is more difficult. To this end the following experiments were performed. Each one of a series of rabbits was inoculated with an overwhelming dose of pneumococci. Then, just as death was imminent in from five to eight hours, the animal was bled to death, and as quickly as possible the blood was defibrinated, the serum passed through a Berkefeld filter to remove the bacteria and the filtrate injected intravenously into a normal rabbit. To our surprise and disappointment, the animals did not die, nor in a second series of rabbits treated in this way were we able to detect any minor harmful effects of such injections.

When one considers the conditions in pneumococcus infection

it is not surprising that there is great difficulty in demonstrating the presence of toxin in the animal, or even of demonstrating the production of toxin by the pneumococcus *in vitro*. The infectious diseases in which active specific toxins have been well demonstrated *in vitro* are diphtheria and tetanus. In these diseases, however, the conditions are unusual. Here a moderate number of organisms growing in the local lesion produce sufficient poison to bring about the most profound intoxication, and it is not surprising that the poison may readily be demonstrated *in vivo* and *in vitro*. In pneumococcus infection, the conditions are different. Even in general infection in the highly susceptible mouse or rabbit, the number of organisms growing in the body is enormous before the animal finally succumbs.

In the severe and fatal cases in man the blood may contain as many as 65,000 organisms per cubic centimetre; and when it is conceived that these are throughout all the body-fluids and the tissues, it is evident what immense numbers of bacteria are responsible for the intoxication and fatal outcome. In man, even when there is no marked invasion of the blood, the number of micro-organisms in the lung must be very large. It is probable that during the course of the disease bacteria are all the time undergoing degeneration, so that from the beginning to the end, large numbers of bacteria have been present. Also the amount of toxin present at any one time may be very small, yet when acting on tissue-cells for six or seven days may produce marked effects.

The suggestion has been made that in pneumonia the symptoms are due to the absorption of products of digestion of the pathological exudate in the lung. It has been well established by various observers that, during the parenteral digestion of protein, substances are formed which may induce fever and symptoms of intoxication. Similar symptoms may be induced by the injection of peptone and other products of protein digestion into the circulation of animals. Most of the work that has been done in the production of fever by means of protein, however, has been carried out with foreign protein and not with the protein of the host. Moreover, at the time the resolu-

tion is probably greatest, that is, following the crisis, such symptoms are not present, although in most cases considerable amounts of the digested exudate are being absorbed. It is hardly likely that the specific and characteristic symptoms of pneumonia can be due merely to the absorption of the products of digestion in the local lesions.

Following the discovery of serum anaphylaxis in guinea pigs and its relation to protein intoxication in man, numerous efforts have been made to bring this into relation to the toxic effects seen in acute infectious disease. Friedberger has shown that if bacteria be treated with immune serum and then with complement, the supernatant fluid, after removal of the bacteria by centrifugalization, will be toxic when injected into a guinea pig, the animal dying within a few minutes with symptoms identical with those seen in anaphylactic shock. He has called the toxic substance produced outside the body in the manner stated " anaphylatoxin," and believes that it is identical with the substance formed within the body which produces the symptoms following the second injection of protein. He thinks that this experiment may offer the explanation for all bacterial intoxications. According to his theory in the period of incubation, during which the bacteria are already present but produce no symptoms, antibodies are being formed, and when these are present in sufficient numbers, the bacterial bodies begin to be split up and the substances so formed produce, not the acute symptoms which are speedily followed by death, because the bacteria are not present in sufficient numbers, but milder symptoms—fever, etc., a kind of chronic anaphylaxis. This can hardly explain, however, what occurs in pneumonia, in which all the evidence seems opposed to a long incubation period, the onset of the symptoms being sudden and apparently the immediate result of the infection.

The production of the so-called " anaphylatoxin " from pneumococci may readily be done, as we and also Neufeld and Dold have shown. Neufeld and Dold,[20] moreover, have shown that similar toxic substances may be obtained from bacteria by simple extraction in salt solution containing lecithin. Rosenow[21]

then showed that if pneumococci are merely placed in salt solution for forty-eight hours at 37° C. (98.6° F.), the extract so formed is toxic, and on injection intravenously into guinea pigs, acute symptoms and speedy death, like those seen in serum anaphylaxis, result. We have studied the effect of the injection of extracts obtained by autolysis in a very large number of guinea pigs,[22] and, in our experience, while occasionally sudden death is produced, this does not occur with great regularity.

Since the salt-solution extracts of pneumococci did not show as high toxicity as was anticipated, it was held possible that in the peritoneal cavity of an animal the solution of the bacteria might go on at a more rapid rate, from which cavity solutions might be obtained of greater and more constant toxicity. Guinea pigs were therefore inoculated intraperitoneally with large doses of pneumococci. As soon as possible after death, the peritoneal cavity was washed out with salt solution. The cells and bacteria were then removed from this solution by centrifugalization and the supernatant fluid was used for intravenous injection into healthy guinea pigs. Of eleven animals so treated, eight showed immediate symptoms like those seen in anaphylaxis, and four of these died within a few minutes with typical features of anaphylactic death and with characteristic necropsy findings.

From the experiments it is evident that the development of the toxic substance is more constant and striking in the peritoneal cavity of the guinea pig than it is in the test-tube. In the animal body, however, conditions are complex and it is difficult to know whether the toxic substance is specific or bears any direct relation to the infectious agent. We therefore tried to obtain solutions of the pneumococcus bodies by other means. Making extracts in chloroform and in ether did not yield solutions that could be readily studied. We next studied the solution of pneumococci obtained by means of bile. In making the solutions a 2 per cent. solution of sodium cholate in normal salt solution was employed. The effect of the intravenous injection of bile extracts of pneumococci has now been tested in a very large number of guinea pigs and rabbits. In a large proportion of cases death with acute symptoms resembling those in ana-

phylactic shock occurs. When smaller amounts of the extract are injected, or when the toxicity of the extract is less, the animals die in from two to twelve hours. Such animals usually show more or less pulmonary œdema and hemorrhages, and small hemorrhages are present in the peritoneum and diaphragm and in the walls of the stomach and intestines.

It is probable, from the effects produced, that the substances operative here are the ones that produce the effects in " anaphylatoxin " and in the salt-solution extracts. In the latter case it has been assumed by Rosenow that the toxic substances result from the digestion of the bacterial protein by the ferments contained in the bacterial cell. The proof of this, however, does not seem convincing. The fact that the solution of the pneumococci in cholate solutions may occur within one-half hour at 4° C. (39.2° F.) is evidence that in this case the active substance is not the result of ferment action. In a recent communication Jobling and Strouse[23] have presented good evidence to show that the lysis of pneumococci in salt solution is probably not merely the result of ferment action. All these experiments indicate that the bodies of pneumococci contain substances which are toxic when they are set free by the solution of the bacterial bodies. They therefore present evidence in favor of the well-known endotoxin theory of Pfeiffer.

During the past few years this theory of the origin of toxic substances has been largely neglected, owing to the interest in the theories of Vaughn and Friedberger, according to which the intoxication in all forms of infection is caused by substances which are intermediate products in the digestion of protein. Vaughn goes so far as to state that the substances producing the symptoms are identical in all infections and that the different symptom-complexes are dependent, not on the nature of the intoxicating substance, but on other conditions. It would hardly seem, however, that the intoxicating substance causing the rapid pulse and rapid, labored respiration and violent delirium of pneumonia is identical with the intoxicating substance in typhoid in which there is a relative slowing of the heart and low, muttering delirium. Though the intoxication

may be due to the products of protein digestion, it does not necessarily follow that the substance is the same in all cases, as the bacterial proteins must differ enormously in composition.

While the obtaining of toxic substances from the bodies of pneumococci is of great interest, it is quite evident that this, in itself, does not contain the proof that we are dealing with the substances responsible for the intoxication in pneumonia. In order to present such evidence, further knowledge is required of the nature of the substance and especially of its relation to pneumococcus immunity.

Certain facts have already been established in regard to this toxin. Its study has been greatly facilitated by the fact that when added to washed sheep-corpuscles hæmolysis occurs. So far as studied, the toxic effects are caused by the same substance which produces hæmolysis, since the two properties are influenced by the same measures and vary in equal degree. One of the most important facts that has been determined in regard to this toxin is that the toxic and hæmolytic properties vary with the virulence of the organism employed. Extracts from non-virulent cultures, so far as studied, are not toxic. The substance which is responsible for the formation of methæmoglobin in the body and the discoloration of blood in cultures, however, does not seem to be present in this toxin. The toxin is destroyed by heating one-half hour at 56° C. (132.8° F.). This may explain why the injection of pneumococci killed by heat produces no effect in the animal injected. It loses its toxicity when kept for twenty-four hours at 37° C. or for two or three days on ice. It may be dried, in which condition its toxic properties disappear much more slowly. It does not pass readily through a Berkefeld filter and it is precipitated by colloidal iron solutions. Many attempts have been made to neutralize its action by the use of dyes, by nucleic acid, nucleates, glycocoll, glycuronic acid, etc., substances which are considered to attach themselves to toxic substances in the body and thus to render them non-toxic. None of these experiments have been successful. The only substance so far found which is able to neutralize the effect of the toxin is cholesterin. When cholesterin is mixed with the

toxic substance and kept at 37° C. for fifteen minutes, the toxic effect, as shown by injection into animals, and also the hæmolytic effect, is lost. When the toxin is mixed with cholesterin and immediately injected into the animal, however, or when the toxin is first injected and is immediately followed by the cholesterin, the toxic effects cannot be prevented. Nor can the toxic effects be prevented by injecting the cholesterin before the toxin. The most important results in this study have been obtained in the attempts to produce antiserums to these toxins, and of these I shall speak later.

Whatever may be the mechanism by which intoxication is brought about, have we any evidence as to the determining factors in the final outcome, that is, as to why the patient recovers or dies? The results of our blood-cultures would seem to indicate that the occurrence of septicæmia plays an important part in the death of the patient. A study of the virulence of the cultures from the blood also seems to show that the intoxication is greater and the prognosis worse when the organisms have a high virulence than when they have a low virulence. Moreover, our clinical experiments seem to indicate that the progressive extension of the local lesion is of bad prognostic import; that the failure of the body to erect a limiting barrier to the local extension of the disease is an important factor in the fatal outcome. At any rate, it is certainly true that in most fatal cases, on examining the lung, one sees, not a sharply localized lesion, but an extending lesion frequently involving several lobes. This progressive extension seems to bear some relation to the virulence of the organism. With organisms of low virulence, the body is able to resist the infection, as regards both the spread of the local lesion and the general infection.

We have made quite extended studies to learn something of the nature of the general resistance of the body to the pneumococcus infection and its effects. It would seem that in pneumonia with its sudden crisis—one of the most startling and dramatic events with which the physician has to deal—an ideal opportunity would be offered to learn the nature of the process of recovery. It must be borne in mind, however, that only in

certain cases does such a critical change in the patient occur. Of about 10,000 cases collected by Musser and Norris[24] the temperature fell by crisis in only about half. In the other cases it is difficult to determine with accuracy just when the change in the patient's condition occurs. It is therefore a mistake to think that in pneumonia we have a sudden change from susceptibility to resistance. More likely the process is a gradual one, and the marked change in the patient's condition occurs when the resisting factor, which increases gradually, reaches a degree sufficient to be effective. This factor of resistance may not be a single one, but the result may be due to a summation of several factors.

It has been suggested that the crisis represents a kind of anaphylactic reaction. It is known that following serum anaphylaxis there occurs a period during which the animal is in a refractory state. If the intoxication in pneumonia is due to peptone-like substances derived from the bacterial protein, it is possible that the crisis is a form of cumulative shock, following which the patient is refractory. Little is known, however, concerning such prolonged anaphylactic intoxication, and the nature of antianaphylaxis is still so obscure that it does not seem profitable to dwell longer on this theory, attractive as it is.

The fact that the crisis usually occurs in about seven days is strongly suggestive that the reaction is a true immunity reaction, since it is about in this time that antibodies appear in the blood in their maximum concentration, as we know from artificial immunization.

The view that recovery in pneumonia is due to the production of immune substances presupposes that at the end of an attack of pneumonia the patient is immune. We know from experience, however, that this is not so, or if immunity is present, it is of very short duration. I have seen a patient return to the hospital with a typical attack of pneumonia two days after discharge from a previous attack. Moreover, it is well known that a person may have repeated attacks; in fact, one attack seems to render a person more susceptible. It is quite possible, however, that the relative natural immunity of man requires

only a very slight assistance in the shape of acquired humoral immunity in order to render the body able to overcome the infection, and following this the immune bodies may very quickly disappear from the blood. The attempts to demonstrate the appearance of known immune substances in the blood during and following an attack of pneumonia have not previously been very successful. An increase of the ordinary bactericidal substances which act in conjunction with complement has not been proved. Most observers have found that the pneumococci grow quite well in the blood-serum of patients recovering from pneumonia, even in the serum of immunized animals.

It has been asserted that by combining the leucocytes and serum of pneumonia patients, or by using the defibrinated blood, definite differences may be demonstrated between the blood of normal persons and that of patients during or following the crisis. None of these experiments seem to me free from objections. There does not seem to be sufficient evidence for the conclusion that the recovery is due merely to an increase of opsonins, though Clough,[25] who has studied the phagocytic activity of the serum obtained after crisis or lysis in a series of eleven cases, found in six of these definite power of the serum to bring about phagocytosis of virulent pneumococci. In two of these cases the serum was tested before crisis and showed no such action. In his experiments, with one exception, the phagocytic activity was limited to the homologous strain. It has been stated by Rosenow that a difference exists as regards phagocytic activity between the leucocytes of patients with pneumonia and those of normal persons, though the results of others (Tunnicliff and Eggers) do not confirm these conclusions. Wolff[26] has attempted to show the increase of phagocytic power in the blood of pneumonia patients by making a composite curve combining the number of leucocytes with the opsonic index. We feel, however, that the errors in the usual opsonic technic are too great to justify his conclusions.

Of more importance are the experiments showing an increased protective power for mice of the blood of patients after recovery from pneumonia, as tested against known lethal doses

of pneumococci. Neufeld has shown that while normal human serum had no protective action, that obtained from certain patients following the crisis had a definite effect. Strouse,[27] and Seligmann and Klopstock,[28] however, failed to demonstrate such changes. Studies on this question were therefore undertaken by Dochez[29] on a series of cases. The method used was the following: Specimens of the patient's serum were obtained on various days both before and following the crisis. When possible, the organism against which the serum was to be tested was cultivated from the patient, either from the blood or sputum. In case the pneumococcus, when isolated, was of low pathogenicity, the virulence was raised by successive animal passages until a dose of 0.000001 c.c. of a broth-culture was sufficient to kill. Twenty-four-hour broth-cultures fresh from animals were used for infection, and the serum and varying quantities of the culture were mixed in the barrel of a syringe and immediately injected intraperitoneally. The appearance of protective substances in the blood could then be detected, as shown by the protocol from one such experiment (Table 1).

TABLE 1.—Protective Power of Serum of an Untreated Patient with Lobar Pneumonia at Varying Stages during the Disease

Quantity of Culture in c.c.	Quantity of Serum in c.c.	Virulence; No Serum	Control; Normal Serum	Serum Obtained								
				Three Days Before Crisis	Two Days Before Crisis	One Day Before Crisis	Three Hours After Crisis	Two Days After Crisis	Four Days After Crisis	Five Days After Crisis	Seven Days After Crisis	Eight Days After Crisis
0.1	0.2	—	—	—	24†	24†	32†	29†	42†	42†	32†	24†
0.01	0.2	—	—	—	42†	20†	75†	42†	42†	27†	—	32†
0.001	0.2	27†	42†	42†	29†	42†	66†	*	*	*	42†	42†
0.0001	0.2	42†	42†	66†	42†	66†	*	*	49†	42†	44†	42†
0.00001	0.2	50†	42†	32†	42†	42†	*	*	*	42†	66†	45†
0.000001	0.2	45†	43†	42†	47†	66†	*	*	*	*	45†	96†

* Animals protected as shown by survival. † Number of hours before death of animal injected.

The serums from fourteen cases of pneumonia were so studied. In ten of these the serums were tested against homologous organisms. Of these ten cases all but one showed at some time the appearance of protective substances in the blood. Of the

serums from four cases tested against stock cultures, only one showed any protective power. The amount of protection was never very high, though in some instances 0.2 c.c. of serum were able to protect against one thousand times the minimal lethal dose. The time of appearance of the protective substances varied somewhat, though in seven instances protective substances either appeared for the first time or showed a marked increase in amount at the time of crisis or, in case of lysis, during the period when the symptoms were abating. In two cases the serum taken during the period of defervescence exhibited little or no power of protection, even against homologous strains, and it was not until some time later, in one case sixteen days, that the presence of protective substances in the blood was demonstrated.

Clough[24] later carried out a similar set of experiments, and in nine out of twelve cases the serums after crisis or lysis showed definite protective power against homologous strains. The technic used differed somewhat from that employed by Dochez and the results were not so striking, but show well that in most cases the serum acquires definite protective power.

These experiments are of great importance as showing, first, that in many cases, at least, protective substances appear in the blood of patients recovering from lobar pneumonia, and second, that these protective substances in many cases are active only against the race of organism concerned in the infection. These experiments, however, do not yet establish that crisis or recovery in pneumonia is due alone to the development of these protective substances in the blood. As already stated, in certain cases they cannot be demonstrated. It is altogether probable, however, that they play some part in the final outcome. As to the nature of the substances which are most active, it is impossible at the present time to state.

Probably recovery from pneumonia occurs when the growth of the organisms is inhibited and their toxic effects neutralized. It is impossible to state which comes first. It is conceivable that if the toxic effects of the bacteria are neutralized, the body is readily able to cause their destruction, since it is possible that

pathogenicity depends entirely on toxicity. There is some evidence, as I shall show, that immune serums are antitoxic. With the present technic it has not been possible to demonstrate increase of antitoxic power of the patient's serum during the crisis.

In the immune-body theory of the crisis, the local lesion is left entirely out of consideration. It is quite evident that in pneumonia we are dealing, not merely with a septicæmia, but with a condition in the lung which has a very important bearing on the termination. The involved portion of the lung forms a solid mass in which are growing numbers of micro-organisms. In each alveolus are fibrin, leucocytes, red blood-corpuscles and bacteria, and in the spaces free serum. Now it is known that as the process advances, the number of leucocytes becomes greater and greater. Resolution finally occurs almost certainly as a result of this increase and associated breaking down of the leucocytes, and with this the setting free of ferments which bring the fibrin into solution. The fact that this does not occur earlier is due to the overbalancing of the leucocytic ferments by the antiferments of the serum, and the lytic ferments become active only when the relation between leucocytes and serum is in favor of the former. It is conceivable that recovery only ensues when such a balance occurs and when, with the solution of the fibrin, tension is relieved and there is an outlet for the exudate. Instead of the surgeon inserting a knife, nature does the work by injecting a ferment.

It is quite probable, moreover, that during resolution other factors than the purely mechanical are at work. With the solution of the exudate, numerous substances are formed which have a direct destructive action on the bacteria. Such substances as the soaps of fatty acids, which are known to have such a destructive action, have been demonstrated in the resolving lung by Lamar.[30] Moreover, it is well known that during the growth of pneumococci outside the body, substances are formed in the culture medium which themselves are destructive. It is quite probable that such substances are being formed in the lung and they may aid in bringing about destruction of the

pneumococci. Against the view that crisis depends mainly on resolution of the exudate, however, may be brought the very evident and conclusive objection that they do not necessarily occur synchronously. Resolution may be long delayed, or resolution may be occurring in one part of the lung while the process is advancing in another.

That leucocytes play some part in recovery is rendered probable by the experiments of Klein and Winternitz.[31] They have shown that when rabbits are treated with benzene, a leucotoxic substance, the animals rapidly succumb to pneumococcus infection, whereas when they are treated with toluene, which is a similar substance but which has no effect on the leucocytes, no decreased resistance is seen. Whether the chief function of the leucocytes consists in limiting the local infection, in which they undoubtedly play a rôle, or in aiding in the development of a general immunity is not indicated by these experiments. Clinicians, however, have long been of the opinion that a low leucocyte content of the blood is unfavorable.

It is not unlikely that in recovery all of the factors mentioned play a part. The destruction of the bacteria in the lung lesion may depend on local factors quite different from those responsible for the destruction of the bacteria in the circulating blood. From present knowledge it would appear that the growth of bacteria in the blood is the most serious part of the pneumonic process, and it seems that this, at least, is influenced by the appearance of circulating anti-bacterial substances.

METHODS OF CURE

It has been known since 1891 that susceptible animals may be rendered resistant to the action of pneumococci by the injection of increasing and properly spaced doses of pneumococci, beginning with the dead organisms. Moreover, it was early shown that if a very small amount of the serum of the immunized animal is injected into a second animal, this animal for a short time is also immune. These experiments are so striking and fundamental that it is no wonder that various attempts have been made to prepare and use such serums

therapeutically. The clinical results, however, have not been convincing. Certain observations made principally by Neufeld and his collaborators, and other observations made in our own laboratory, suggest reasons why such results have not been satisfactory and methods for overcoming the difficulties.

Opinions have differed as to whether or not an immune serum produced by the injection of a given race of pneumococci into an animal is effective against all races of pneumococci. The first accurate studies on this problem were made by Neufeld and Händel.[32] They tested a so-called univalent serum against various races of pneumococci. While this univalent serum was protective in mouse experiments against fifteen strains studied by them, against other strains the serum had practically no effect. They decided that these atypical strains were not *Serum-fest* in the ordinary sense of the term, since the serum obtained during convalescence from one of the patients, from whom one of these organisms had been isolated, protected mice against the homologous strain and also against one of the other atypical strains, but did not protect against the typical strain. They then produced an immune serum against one of the atypical strains to see whether all atypical strains could be affected by this immune serum, but found this not to be true. In their further studies they found that the second immune serum, which they called *Serum Franz*, protected against only three of the atypical strains isolated by them, but failed to protect against three other strains. These latter three strains they further showed to be individual in their reactions. Neufeld and Händel did not have access to a large number of patients with pneumonia from whom to obtain cultures, and could not determine the frequency of occurrence of atypical types, nor could they make extended studies on grouping of the organisms on a biological basis, though from their studies the possibility of making such a grouping was most evident.

With the opening of the Hospital of the Rockefeller Institute in October, 1910, patients suffering from lobar pneumonia were admitted for treatment and study, and an extended study was commenced of the pneumococci obtained in these cases. An

immune serum was prepared by injecting a horse with a culture of pneumococcus obtained from Professor Neufeld, the same race he had employed in the production of his immune serum. The protective power of this serum for mice was tested against a number of races of pneumococci cultivated from a series of cases of pneumonia. A report by Dochez[33] gave a preliminary report of this study, indicating that this serum protected against only about half the races studied. It was therefore evident that if such a serum were employed therapeutically, an effect could be expected at the most in only about half of the cases treated.

Experiments[34] were then undertaken to determine whether it would be possible to make a biologic classification of pneumococci obtained from cases of pneumonia, based on their reaction to different serums in protection experiments. Rabbits were therefore immunized to each of the races which were not acted on by the horse-serum, which we have called Serum 1, and the protection afforded by these different rabbit serums against all the other races tested. A considerable number were found to show cross-protection, that is, a serum prepared by injection of one of the number acted on all the races of this group. A horse was then immunized to one of this group and the serum is called Serum 2. In this way it has been possible to divide the pneumococci obtained from cases of pneumonia into four groups. In Group 1 are included all the races against which Serum 1 is effective. In Group 2 are included all those against which Serum 2 is effective. Whether the races included in this group correspond with the organisms described by Neufeld as acted on by his immune serum *Franz* is not known at present. In Group 3 are placed all the organisms of the so-called *Pneumococcus mucosus* type. These organisms have very large capsules and produce a sticky exudate in animals. In Group 4 are included all the races against which Serums 1 and 2 are not effective and which, from their other properties, do not belong in Group 3. Animals may readily be immunized to any member of this Group 4, and the serum of the immunized animal is protective against the race used for immunization. In no instance, however, has this serum been found effective against any other

race of this group or against the organisms of the other groups. So far as cultural and morphologic characters are concerned, no constant group differences have been discovered between the members of Groups 1, 2 and 4. By means of the agglutination reaction, however, it has been found possible to group them in exactly the same manner as by protection experiments.

As previously stated, the members of Group 3 differ from the others somewhat in their morphologic and pathogenic characters. They differ further in the fact that while animals may be very highly immunized to them, the serum of such animals possesses no protective power; they induce active but no passive immunity. Studies have been undertaken by Hanes to learn

TABLE 2.—Agglutination of Pneumococcus Mucosus

Organism No.	Immune Serums Nos.								Normal Rabbit Serum
	19	26	42	54	68	96	I	II	
19	+	+	+	+	+	+	−	−	−
26	+	+	+	+	+	+	−	−	−
42	+	+	+	+	+	+	−	−	−
54	+	+	+	+	+	+	−	−	−
68	+	+	+	+	+	+	−	−	−
96	+	+	+	+	+	+	−	−	−
I	−	−	−	−	−	−	+	−	−
II	−	−	−	−	−	−	−	+	−

on what factor this failure to produce passive immunity depends. It was found that the serum of the immunized animals not only does not protect, but also has no agglutinating power. It has been known that certain encapsulated bacilli also fail to be agglutinated by immune serum. Porges,[35] [36] however, has shown that such bacilli are agglutinated by the serum of immunized animals, provided the bacilli are previously treated so as to destroy their capsules. This method was therefore employed by Hanes[37] in studying these cocci. Six typical races of *P. mucosus* obtained from cases of pneumonia were studied. The bacteria were treated with dilute hydrochloric acid and heated for fifty minutes at 80° C. (176° F.). The fluid was

then neutralized and the bacteria so treated tested for agglutination. Controls were made with members of Groups 1 and 2 treated in the same way. The results were definite and striking. Agglutination of all the six races of *P. mucosus* occurred promptly with all six immune serums obtained by inoculating each of a series of rabbits with one of these races. No agglutination of Pneumococcus 1 or 2 occurred with any of these serums, and *P. mucosus* was not agglutinated by either Serum 1 or 2. (See Table 2.)

These experiments show that, so far as tested, all the organisms of the *P. mucosus* type belong in one biological group differing from those of the other groups. In order to show the relation of these organisms to streptococci, the method of complement-fixation was employed. With this method there occurred a considerable amount of cross-fixation among the various races of pneumococci, including *P. mucosus*, but no cross-fixation was observed in testing the complement-fixing powers of *Streptococcus mucosus* or *S. pyogenes* serums. It therefore seems evident that *P. mucosus* is really a variety of pneumococcus, and that biologically it forms a distinct variety of this organism.

Further studies of the various members of the *P. mucosus* group, to see if any were affected by the immune serum *in vivo*, were all negative. None of the serums were able to protect mice, even against the homologous organism. These experiments and also observations of Gruber and Löhlein seem to indicate that the failure of such serums to protect is in some way related to the formation of the thick, mucoid capsules by these organisms. As soon as the bacteria commence to grow in the body, capsules are formed which prevent the action of the immune serum. By the methods employed by Dochez and Hanes, it has been possible to study the races of pneumococci obtained from a series of cases of pneumonia. The classification by protection and agglutination experiments of sixty-two organisms so obtained gave results as shown in Table 3. In every instance in which an organism could be placed by protection in one of the groups described, the agglutination reaction has corresponded.

The races placed in Group 4 have been called heterogeneous, since each race, so far as studied, appears independent, and no grouping of the members on a biologic basis, by means of protection or agglutination, is at present possible. Table 4 shows the results of the study of agglutination with these races.

Gillespie[38] has also made a study of the various races, using the method of agglutination of bacteria by acid, as introduced by Michaelis. The results also show certain group differences in the agglutination of the various races.

Recent observations by Rosenow indicate that by certain methods it is possible completely to change the characters of the organisms of the entire streptococcus-pneumococcus group, so

TABLE 3.—Classification by Protection and Agglutination

	Number	Per Cent.
Group 1	35	47
Group 2	13	18
Group 3 (P. mucosus)	10	13
Total typical	58	78
Group 4 (heterogeneous)	16	22
Total heterogeneous	16	22
Total number	74	

that one may be transformed into the other, even *S. hæmolyticus* into a typical pneumococcus and *vice versa*. It has long been thought that the various closely related bacteria must originally have had a common source and have become differentiated by processes of adaptation. It is remarkable, however, that the changes can occur in such a short period of time as shown by Rosenow, even though they are subjected to extreme changes in environment, as has apparently been done. Some experiments performed in my laboratory several years ago by Strouse indicated that sudden mutations might appear in this group. Important as these experiments are, they do not have an immediate bearing on the pneumonia problem, except as regards the origin of the infection.

As regards the course of the disease and the possibility of specific methods of cure, the possibility of transformation of type of the organism concerned is not significant. In all the studies of organisms obtained at different times from the same case, in no instance have there been any indications of a change in type; the type first isolated has always subsequently been found. Moreover, many of these strains have now been cultivated for a long time outside the body, both in artificial mediums and in repeated passages through animals, some of them for several years, and they have in all cases retained their original characteristics. The results of the present year are not included in Table 3. This year the largest number of cases has been due to organisms of Type 2. It is possible that the prevalence of cases due to the various types varies from year to year and in different places, which may explain the variation in mortality observed in different times and places. The mortality of our limited number of cases due to organisms of different types is shown in Table 5. The most striking fact is the low mortality due to organisms of Type 4. Further observations may possibly change our ideas with regard to the relative severity of cases due to organisms of the different types.

TABLE 4.—Strains of Heterogeneous Group Tested and Results of Agglutination

Serums Pneumococcus	Pneumococcus Strain Numbers												
	1	2	34	36	37	38	52	55	60	62	71	76	82	102
1	+	0	0	0	±	0	0	0	0	0	0	0	0	0
2	0	+	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	+	0	0	0	0	0	0	0	0	−	0
37	0	0	0	0	+	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	+	0	0	0	−	0
71	0	0	0	0	0	0	0	0	0	0	+	0	−	0
76	0	0	0	0	0	0	0	0	0	0	0	+	0	0
82	0	0	0	0	0	0	±	0	0	0	0	0	+	0
Polyvalent, 34, 38, 62	0	0	0	0	0	+	0	+	0	+	0	0	−	0

In addition to the fact that there are immunological differences in the pneumococci concerned in pneumonia, there is

probably another reason why the use of immune serum has not proved efficacious in the past. The method of employment of such immune serum has been to use small doses, from 10 to 20 c.c., usually given subcutaneously. Neufeld and his assistants, by titrating immune serum against varying doses of pneumococci and making injections into mice, have concluded that in order to obtain protective power a certain proportion of serum in relation to body-weight is required. This concentration they have called the *Schwellenwert* or threshold concentration, and from experiments on mice they estimate that for man the dose of serum employed by them must be at least 77 c.c. Undoubtedly, however, this *Schwellenwert* must vary enormously under different conditions, depending on the virulence of

TABLE 5.—Mortality in Patients Infected with Varying Types of Organisms

Infection Type	No. Patients	Patients Died	Per Cent.
1	34	8	24
2	13	8	61
3	10	6	60
4	15	1	7
Total.....	72	23	32

the organism, the time the serum is given, etc. In any case their experiments indicate that the serum must be given in very much larger amounts than has hitherto been done, in order to obtain curative results. They concluded that such an antibacterial serum does not obey the law of multiple proportions. This is undoubtedly true for the conditions employed by them, namely, injections made separately in different parts of the body, and is also true for the conditions present in the therapeutic injections in man. Dochez has shown, however, that when the serum and cultures are mixed before injection, such a serum does obey the law of multiple proportions up to a certain point, but there is a maximum degree of infection against which no amount of serum, however large, is able to protect.

It would therefore seem that one of the factors of the protective mechanism must be supplied by the body, and that, when the infection is very great, a sufficient number of immune bodies may be supplied by the administration of serum, but the body cannot react to a sufficient extent adequately to supply this second factor. This suggests that in order to obtain results from serum, it should be administered early, before the infection has reached too extreme a grade, beyond which no amount of serum can be effective, and also offers a possible explanation of the fact that in certain cases, such as the one which I shall mention, Case 4, the serum seems to have absolutely no effect. The nature of this additional factor is not known. If, as previously stated, the serum owes much of its effect to its bacteriotropic power, the number and activity of the leucocytes may be the additional factor. That this factor may be stimulated is shown by the results of artificial immunization, whereby very much larger doses of bacteria are resisted than can be protected against by immune serum.

These experiments have indicated that for the successful employment of immune serum in pneumonia, it must be employed fairly early and in large doses, and a serum must be used which is effective against the variety of organism causing the infection. We have been able to produce a serum of very great efficiency against organisms of Type 1, and one effective against organisms of Type 2. So far it has been impossible to produce a serum effective against *P. mucosus*, and, for the reasons stated, it would only be practical to treat cases of pneumonia due to organisms of Type 4 with homologous serums. This is of less importance, however, since the cases due to these organisms are apparently of mild grade.

To employ the serum effectively in cases due to organisms of Types 1 and 2, it has been necessary to devise a method for quickly determining in each case the type of organism concerned. This has been done and the following method is employed. When a patient with pneumonia is admitted to the hospital, a culture is immediately made from the blood and also one from a portion of sputum coughed up from the lung, or,

when this is not obtainable, a culture is made directly from the lung by the insertion of a needle. This procedure seems to be without danger. When there are large numbers of organisms in the sputum, a culture may be obtained most rapidly by injecting the washed sputum into the abdominal cavity of a mouse. After four or five hours the peritoneal cavity may be washed out with salt solution and the cells thrown down in the

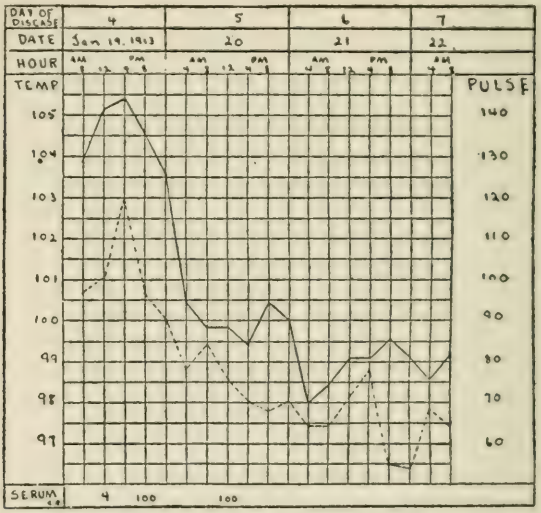

Fig. 1.—Case 1, E. S., No. 988, aged 20 years, admitted January 18, 1913, on the third day of the disease. Physical signs showed involvement of the lower left lobe and also signs of beginning involvement in the lower right lobe. Blood culture was negative. Pneumococci obtained from sputum were of Type 1. The patient was quite ill on admission, respirations were labored and there was cyanosis. His condition was markedly improved on the day following the first administration of the serum, as shown by the temperature and pulse curves. Recovery was uneventful except that slight signs in the lungs persisted for several weeks.

centrifuge; a suspension of the organisms is thus obtained. In whatever way the culture is obtained, the agglutination test is at once applied. If the organism fails to agglutinate with either Serum 1 or Serum 2, it is, of course, useless to undertake serum treatment. If, however, one of the serums agglutinates the organism, treatment may be commenced at once with the appropriate one.

So far it has been possible to treat only a comparatively

small number of cases. Twenty-three cases have been treated with the serum. Of these, fifteen were due to organisms of Type 1 and eight to organisms of Type 2. The method of administration of the serum was the following: When admitted, the patient was given 0.5 c.c. of serum subcutaneously to discover if hypersensitiveness existed. As soon as the type of organism was determined, from 50 to 100 c.c. of the serum, diluted one-half with salt solution, were injected intravenously. The condition of the patient served as a guide in the later treatment. Usually the serum was not administered oftener

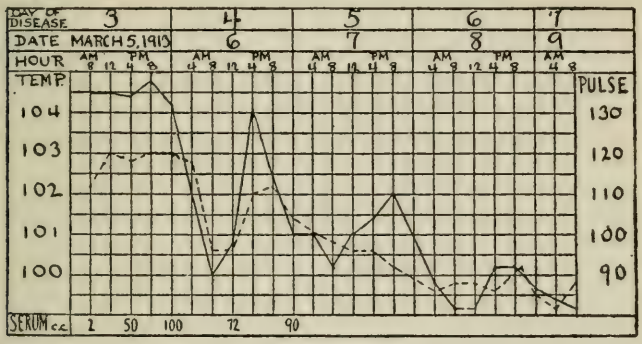

Fig. 2.—Case 2, B. G., No. 1175, aged 36 years, admitted March 4, 1913, on the second day of the disease. There was slight involvement at the base of the right lobe. Blood culture was positive. Agglutination test showed Type 1 organism. Treated with serum on day following admission. The signs of involvement in the right lower lobe became more distinct, but there was no apparent extension of the involvement beyond this lobe during the course of the disease. Following injection of the serum the patient's general condition improved. The patient complained of some urticaria and joint pains beginning on the twelfth day.

than every twelve hours. The patients treated received totals of from 190 to 460 c.c. of serum, except one, who received a total of 700 c.c. of serum. The patients treated were all seriously ill. They were treated in series. Every case infected with a pneumococcus of Type 1 or Type 2 was treated. Of the fifteen cases due to Pneumococcus 1, all of the patients recovered but one, and of the eight cases due to Type 2, two patients died. One of these patients objected to the treatment and would not allow its continuation, so it was not thoroughly carried out. When we consider that the mortality among the

untreated patients infected with Types 1 and 2 is very high (Table 5), the result is certainly not discouraging. It must also be remembered that so far most of our cases have been admitted late in the disease. Treatment was commenced on the third day in six cases, on the fourth day in five cases, the fifth day in six cases and the sixth day in six cases. If treatment can be commenced early, it is probable that the results will be even better than they now are. It is to be hoped that during this winter a large number of cases may be treated early in the disease. Effective treatment in the cases due to Types 1 and 2 should cut down the total mortality due to pneumonia very materially, as it has already done in our hands. I prefer at present, however, not to lay the main stress on the mortality statistics, since these are not large enough to be conclusive, but to refer to other criteria which we possess as to the efficacy of the serum.

Let us first consider the effect on the clinical course of the disease. Following practically all the injections, a reaction has occurred. The temperature usually rises and then falls, but does not necessarily remain low. In two instances the rise of temperature has been marked. In the other cases the rise of temperature following the injection was only a degree or so. In all the cases except the fatal ones, the serum has apparently had an ultimate favorable effect in lowering the temperature and shortening the course of the disease, though, of course, this is a very difficult matter of which to be absolutely sure. In no case was one injection of the serum sufficient to bring on a crisis.

Figures 1, 2, 3 and 4 show the effect of treatment on the temperature curves in certain of the cases. It is manifest that wrong impressions may be produced by the exhibition of temperature curves unless all the curves of a series are given. To avoid this difficulty, so far as possible, however, a curve from each group of cases is shown. Figure 1 represents the curve of a case in which the serum apparently had a marked effect, the temperature falling promptly and in a striking manner. Figure 2 indicates a temperature curve in a case in which

the temperature fell following the administration of serum, but several doses were necessary before the temperature remained low. Figure 3 shows the temperature curve in a case in which there were apparent effects of the administration of serum, but after the administration of serum was discontinued, the temperature curve rose and only fell after further large doses of serum were administered. In Figure 4 is given a temperature curve in which the serum had apparently no effect and the patient died on the seventh day.

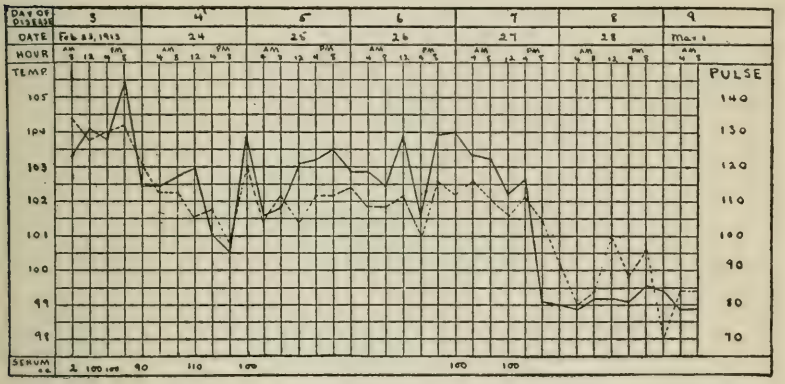

Fig. 3.—Case 3, M. L., No. 1091, aged 30 years, admitted February 22, 1913, on the second day of the disease. Blood culture showed a growth of pneumococcus, Type 1. Physical signs indicated involvement of the right lower lobe with slight involvement of the left lower lobe. The patient's condition improved following the treatment and the signs in the lungs became less well marked. On the sixth day, however, the patient's condition was again worse, the temperature was higher and the pulse more rapid, so that treatment was commenced again, following which the patient's condition markedly improved and he made a rapid recovery except for symptoms due probably to serum sickness on the twentieth to the twenty-fifth days of the disease.

All the patients seemed to feel better following the injection of the serum, and in a number of cases the apparent lessening in the degree of intoxication was very manifest. When the treatment was commenced early, no extension of the involvement of the lung occurred. On the other hand, there was no special tendency in the treated cases for the lung lesion to resolve rapidly. If anything, there seemed to be a tendency for resolution to be delayed in these cases. This has been noted by others in certain cases treated by serum.

More important than the foregoing criteria, however, as indicating an effect of the serum, are the following observations, since they have depended solely on objective procedures: First to be mentioned is the effect of the serum on the organisms in the blood. In ten cases pneumococci were isolated from the blood before the treatment was commenced. In all cases blood-cultures were made before each treatment; and in all of these ten cases after one treatment and before the second (or within

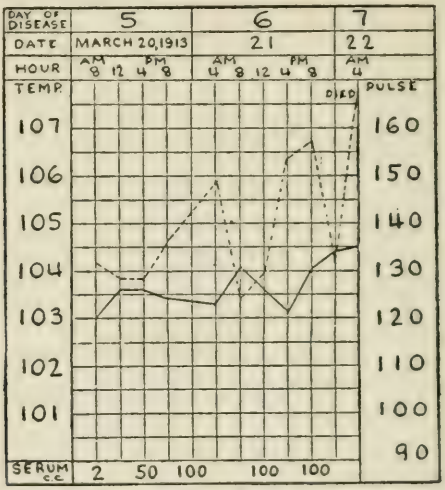

FIG. 4.—Case 4, F. W., No. 1090, aged 36 years, admitted March 19, 1913, on the fourth day of the disease. The patient was extremely ill on admission. There was involvement of the right upper and lower lobes. Blood culture and culture from the lung showed the presence of pneumococcus, Type I. There was some irregularity of the heart. Following treatment on the fifth day, the patient seemed better. The blood culture was negative. The pulse continued rapid and showed an extrasystolic irregularity, however, and the patient showed a very marked cyanosis. In spite of the active serum treatment, the patient died on the seventh day.

from eight to twelve hours) the blood had become sterile. The conclusion seems justified, therefore, that one large dose of active serum given intravenously is sufficient to sterilize the blood. It also seems certain that if the organisms are not present in the blood, the administration of the serum will prevent their invasion. Second, in a previous study of the protective substances in the blood-serum of patients with pneumonia, it has been shown that, as a rule, the appearance of

protective substances in the blood, when demonstrable, coincides rather sharply with the period of critical fall in temperature and the disappearance of symptoms. Before the crisis they are not present in the blood in any measurable degree.

A similar study has been made by Dochez of the protective substances in the serum in a number of the cases of pneumonia treated with serum. In all the cases studied, it has been possible to demonstrate the appearance of such substances in considerable amounts in the serum very shortly after the administration of one dose of the immune serum, even when this serum has been administered early in the disease, at a period when such protective substances are otherwise never present (Table 6). These

TABLE 6.—Protective Power of Serum B. G.; Treatment Commenced on the Third Day

Quantity of Culture in c.c.	Virulence; No Serum	Quantity of Serum in c.c.	Serum Obtained			
			Third Day, Before Treatment	Third Day, 6½ Hours After Treatment	Fourth Day, After Treatment	Ninth Day, Five Days After Last Treatment
0.001	—	0.2	16†	*	*	† Days
0.0001	—	0.2	20†	† 5 Days	*	*
0.00001	24†	0.2	24†	*	*	*
0.000001	28†	0.2	24†	*	*	*

*Animal protected as shown by survival. †Time in hours before death of animal injected.

substances persist, and in case they play a part in the mechanism of recovery, as was concluded from the previous study, it is evident that their appearance indicates a favorable action of the immune serum.

The results obtained, therefore, from the clinical and laboratory study of this series of cases of pneumonia treated by the injection of large amounts of appropriate serum, seem to indicate that a method has been devised for the successful specific treatment of at least a portion of the cases of acute lobar pneumonia. Studies on the treatment of pneumonia by the intravenous injection of the Neufeld-Händel immune serum have been made by Beltz,[39] Weitz[40] and Geronne.[41] In none of these

series, however, were studies made of the type of organisms concerned in the infection of the cases treated, and in all of the cases the amount of serum administered was too small, judging from our own experience, to be of value.

The mode of action of the immune serums is still somewhat obscure. It is quite evident that there is an antibacterial action, inasmuch as the bacterial invasion of the blood is prevented. The action on the local lesion, however, is less evident. It is probable that here the organisms can less readily be reached by the serum, though apparently in most cases the growth of the bacteria in the margins of the lesion has been inhibited, as shown by prevention of spread of the process. In addition to the antibacterial action, the clinical cases show a definite change as regards intoxication. It is possible, of course, that this is entirely associated with the destruction of the organisms. Certain experimental work, however, has indicated that the serum may possess some antitoxic effect.

When the immune horse-serum is added to the toxin obtained by dissolving pneumococci in bile, it is found that such a serum has a well-marked effect in inhibiting the hæmolysis of sheep corpuscles by this toxin. When it is added to the toxin in doses of 1 c.c. of serum to 4 c.c. of toxin and placed at 37° C. (98.6° F.) for one-half hour, the effect of the toxin when injected into guinea pigs is diminished or entirely prevented. These effects of the immune horse-serum are much less specific, however, than are the protective or antibacterial effects, since the Serum 1 acts on both Toxins 1 and 2, though most markedly on Toxin 1. Serum 2 also shows a similar diminution in specificity in antitoxic action as compared with antibacterial action. These experiments offer some evidence that part of the effect of the immune horse-serum is antitoxic, admitting, of course, that the toxic substances obtained from the bacterial bodies are responsible for the intoxication.

An effort has been made to obtain a pure antitoxic serum by the injection of the toxin alone into animals. Rabbits have been immunized by the repeated injection of this toxin and a sheep also has been highly immunized. The sheep-serum and also the

immune rabbit-serums show antitoxic power, as indicated by antihæmolytic action and also by neutralizing effect on the toxin, as tested by injection into guinea pigs. The effects, however, are less marked than those of the antibacterial horse-serum. These antitoxic serums are also protective against the living organisms, as shown by tests on mice. The protection, however, while fairly high, is less well marked than that of the horse-serums. The protection is not so specific as that of the horse-serum, since the serum produced by the injection of toxin prepared from an organism of Type 1 is not only protective against this organism, but also, though to a less extent, is protective against organisms of Type 2.

The interpretation of these experiments is attended with much difficulty. It is possible that these antitoxic serums may show protective power only because living organisms were introduced, since in the preparation of the toxin one cannot be positive that all organisms have been destroyed. These antitoxic serums, however, possess no power to cause agglutination, and this fact, together with their lessened specificity, suggests that we are dealing with serums which owe their power to other properties than those of the antibacterial serums. The experiments are of importance, moreover, since they indicate that immunity may be obtained against the substances contained in the bile extracts, and since the essential criterion of a toxin in the Ehrlich sense is that immunity may be obtained to it. Much more work will have to be done before such antitoxic serums should be employed therapeutically.

It is probable that in the future it will be possible to obtain the same therapeutic effects by the injection of much smaller amounts of serum than are now employed. Work now being carried on by Avery shows that the immune substances are all contained in the globulin fraction of the serum, and methods are now being devised for the concentration of the serum, so as to avoid the injection of a very large part of the serum protein which contains no immune substances. In this way it will probably be possible to avoid serum sickness, which has occurred in a number of our patients in from ten to twelve days after

the administration of the large amounts of horse-serum. This serum-sickness, while causing some discomfort to the patients, is not of any serious import, so far as we know.

It may be possible later to produce polyvalent serums that are efficacious. At present, however, and until the value of the special serum in the cases due to organisms of Groups 1 and 2 is unquestionably determined, it does not seem to be advisable to make such attempts. The objection is frequently raised that this method of treatment is very complicated. One may reply to this that so is the treatment of appendicitis.

At the present time I can do no more than mention the efforts along other lines that have been made to produce curative results by specific measures. Most important studies were made by the late Professor Hiss in the treatment of bacterial inflictions by means of leucocytic extracts. So far as concerns pneumonia, the results of experiments on animals are not very convincing, but the brief clinical report of cases of pneumonia treated, as stated in the article published since his death, seems extremely favorable and promising. It is to be hoped that study along this line will be continued.

Lamar has devised a method for the local treatment of pneumococcus infections. He has shown that immune serum has a much greater effect on pneumococci treated with sodium oleate solutions than on cocci simply washed in salt solution. This action of the soap, however, is inhibited if the serum be added first or mixed with the soap solution before treating the bacteria. The inhibiting action of serum, however, may be prevented by the addition of small amounts of boric acid, as Liebermann and von Fennyvessy have shown. By combining the soap, serum and boric acid in proper concentrations, Lamar has found a mixture that is much more efficacious in the local treatment of experimental pneumococcus infections than is serum alone. The treatment of local infections, as meningitis, with such a mixture, using serum effective against the race of organisms concerned, should be tried in all suitable cases. It is doubtful, however, whether such a mixture can be employed intravenously.

A final possible method which may be rendered practical in the treatment of pneumonia is along the lines of chemotherapy, as laid down by Ehrlich. It has been generally held that such a method of treatment may be of value in protozoan infections, but not in diseases due to bacteria. Morgenroth[42] and his co-workers, however, have shown that a derivative of quinine—ethylhydrocupreine—has a specific action on pneumococcus infections in mice, and Wright has shown that this drug is bactericidal for pneumococci in the test-tube. The drug has been employed clinically, but cases of amblyopia developing have indicated that the toxic dose in man too closely approaches the curative dose to permit the safe administration of the drug. It is possible, however, that with further study, its toxic properties may be reduced without lessening its curative effect.

CONCLUSIONS

Much obscurity still exists concerning the mode of natural infection in pneumonia, though by animal experimentation many facts in regard to it have been discovered.

The symptoms in pneumonia are probably due to toxic substances derived from the bacterial cells.

The outcome is dependent on the virulence of the organisms concerned and on the ability of the body, first to limit the local infection, and second, to prevent the invasion of the blood by the organisms, as on the latter the outcome of the disease mainly depends.

Leucocytes probably play a part in the resistance, certainly as regards the local spread, and probably also to some extent as regards the general infection.

The most important part in prevention of the general infection is probably played by immune substances contained in the serum. Such substances are present in the serum of immunized animals.

Pneumococci differ in regard to their immunological reactions and on these they may be divided into several groups.

In order to use immune serum effectively in treatment, as in prevention, it is necessary to employ the serum effective against

the group of organisms to which the special organism causing the infection belongs.

Immune serums effective against two of the most important groups have been produced. This treatment has been carried out in a limited number of patients with promising results.

It is probable that the methods of application of such serums will be improved, and it is possible that the method may be combined with other measures directed toward other factors which are important for the outcome. In any case, facts regarding the nature of the disease are being disclosed, and the outlook, at least for lessening the ravages of this dreadful disease, is encouraging.

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THE PHENOMENA OF INFECTION*

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DOERR of Vienna (Handbuch d. path. Mikroorganismen, Zweite Auflage) closes a most excellent review of recent work on so-called anaphylaxis or protein sensitization with the following paragraph:

> While it must be admitted that the action of those infective bacteria, which are not known to produce specific toxins, remains without explanation, and while the theories which have been developed by Von Pirquet, Friedberger, Vaughan, Schittenhelm, Weichardt and others have opened up a new way to the understanding of incubation, fever and crises, still it must be borne in mind that the premises of these theories do not possess the force of chemical facts. It has not been positively shown that the symptoms of anaphylaxis are due to the parenteral cleavage of proteins, that the true anaphylactic poison is identical with that produced *in vitro* and that both come from the antigen. Even if we agree with Dold, Sachs and Ritz that so far as the rôle of anaphylaxis in the infectious diseases is concerned, it is irrelevant from what matrix and by what processes the hypothetical anaphylactic poison is produced, even then all the difficulties are not removed. Numerous infecting agents are not anaphylactogens; they do not differ in their effects upon sensitized and non-sensitized animals and even when there are differences they are slight compared with those seen when the protein antigens derived from the higher plants and animals are employed. The relatively simple structure of the bacterial proteins is the cause of this. Therefore it is questionable whether one has the right to explain the phenomena of the infectious diseases with serum sensitization as a starting-point. Moreover, the infections are not so monomorphic as some suppose from a superficial consideration. Measles and scarlet fever seem much alike, still during an attack of the former the body cannot be sensitized to tuberculin or vaccine, while the latter does not induce this condition.

This statement, following as it does a fair but critical review of the new theories referred to and the work upon which

* Delivered January 17, 1914.

they rest, is certainly just. The purpose of this paper is to state the sensitization or parenteral digestion theory, as I understand it, and weigh the evidence for and against it. It should be plainly understood that in doing this I am speaking my own views and it is probable that no one of the investigators with whom Doerr has done me the honor of grouping me would agree with me in all details, nor I with them.

It may be well to meet at the outset the chief objection which Doerr makes, in the above quotation, to the sensitization theories. He states that the bacterial proteins have a relatively simple molecular structure. This is an assumption without a fact to support it. Because bacterial cells are relatively simple morphologically it has been inferred that they are simple in the chemical structure of their protein constituents. This certainly is not true. My students and I have shown that the bacterial proteins are quite as complex as those of the cells of our own bodies. They contain at least two carbohydrate groups, one of which has been quite positively located in the nucleic-acid fraction, while the seat of the other has not been determined. They furnish nuclein bases, thus showing the presence of one or more nucleic acids, as has been inferred from their behavior toward the basic stains. They yield diamino- and monoamino-acids in like abundance and variety as these are found in the proteins of the higher plants and animals. In short, bacteria consist largely of glyconucleo-proteins. Recent papers from Kossel's laboratory (Zeitschrift f. physiolog. Chemie, 1913, lxxxix, 85) confirm our claim that the chemical structure of the bacterial proteins is not simple.

Doerr's statement that numerous infective agents are not anaphylactogens is one which I can neither confirm nor deny. It seems to me, however, to be an assertion which needs qualification. There are many kinds and degrees of sensitization depending upon the sensitizer and the cell acted upon. In some instances the sensitized state continues for many years; in others it lasts for only a few weeks or months, while in still others it is even more ephemeral.

In order to save space and time I will formulate my views concerning the phenomena of infection as follows:

All infecting agents are living proteins capable of growth and multiplication. They may contain carbohydrates, fats and waxes, possibly other and simpler chemical bodies, but their essential and characteristic constituents are proteins. This is true not only of all infecting agents but of all life units. The infecting agents which we know are bacteria, protozoa, moulds and yeasts. These possess physical characters which enable us to individualize them, but theoretically there is no reason why a living thing, and consequently a virus, may not be a liquid. Each and every living thing must feed, assimilate and excrete. Its molecules must be in a labile state, taking in and casting out atomic groups simultaneously. The bacterium can feed only on the pabulum within its reach and of that it can utilize only that which it can fit into its molecular structure. Only this is pabulum to the cell. Organisms which cannot utilize the proteins of the animal's body cannot be pathogenic to that animal. All living things feed through the activity of their ferments. These are of two kinds, analytic and synthetic. The former split up the pabulum into proper building stones, while the latter place these stones in proper position in the cell molecule. Usually we say that cell ferments are extra- and intracellular. The former diffuse more or less into the medium and exert a cleavage action; the latter remain in the cell and do a constructive work. That these ferments are in reality different bodies is indicated not only by the parts they play in the life of the cell, but also by the fact that they are differently affected by heat and chemical agents. Ferments are specific in two senses; first, each kind of cell elaborates its own ferment; and second, the ferment is able to split up only certain proteins. Besides, for each ferment there is an optimum temperature at which its action is greatest. There are many bacteria which cannot grow at body temperature. These organisms cannot be pathogenic. This is true of most saprophytic bacteria found in water. Then, there is the relation between ferment and substrate which requires a nicety of adjustment which is not

thoroughly understood. Finally, in a general way an accumulation of fermentative products retards the action of the ferment.

It must be remembered that the body-cells, like the bacterial cells, digest proteins. They also elaborate analytic and synthetic, or extra- and intracellular ferments. These ferments have been especially studied in the leucocyte. The extracellular ferments elaborated in leucocytes are germicidal because they digest bacterial proteins, and they are destroyed by a temperature of 56°. The intracellular ferments of the leucocyte are also bactericidal and for a like reason, but they bear a much higher temperature. Every living cell in the animal body, like every bacterial cell, elaborates its specific ferments. This has been positively demonstrated by Abderhalden and his students (Zeitschrift f. physiolog. Chemie, 1913, lxxxvii, 220, 231).

It must be evident from what has been said that the pathogenicity of a given virus is determined by its ability or inability to grow in the animal body. Its inability to do this may be due to the fact that it cannot digest and therefore cannot feed upon the proteins of the body or it may result from the fact that the ferments of the body-cell do digest and destroy the bacterial proteins. Herein lies the explanation of all forms of bacterial immunity either natural or acquired. Toxin immunity is quite a different thing and will not be treated in this paper.

In case of exposure, the chance of infection depends upon several variables, such as the number and viability of the organisms introduced and the state of health or capability of resistance on the part of the animal. In man the effectiveness of the defensive ferments is influenced by heredity, age, food and possibly other conditions. The great fatality of measles and tuberculosis among those peoples who have inherited no resistance to these diseases is well known. That infants and adults are physiologically protected to a marked degree against diphtheria while children are largely without protection has been demonstrated by Shick (Münch. med. Wochenschrift,

1913, p. 2608) and others. We have long known that typhus, plague, beriberi, scurvy and pellagra are most in evidence when abundance and variety in food are lacking, and the work of Osborne and Mendel (Jour. biolog. Chem., 1913, xv, 311), McCollum and Davis (ibid., xiv, 40), Wellman and Bass (Jour. Trop. Med., 1913), Funk (Münch. med. Wochenschrift, 1913, p. 2614) and others on the vitamines promises much.

When the infecting organism multiplies rapidly and soon leads to general sensitization of the body-cells, the disease developed is acute. On the other hand, when the invading organism finds the conditions for its growth less favorable, it multiplies slowly and only imperfectly and locally sensitizes the body-cells, the disease is chronic. When the virus is widely distributed throughout the body and sensitization is also general, the disease is systemic. On the other hand, when the virus and sensitization are restricted the disease is local. In cattle and sheep the anthrax bacillus grows rapidly, becomes abundant in the blood, sensitizes generally and consequently develops an acute systemic disease. On the other hand, in the hog the growth of the anthrax bacillus is restricted to the lymphatic glands, sensitization is equally local, and the disease is both local and chronic.

That a given pathogenic bacterium may grow in one animal and not in a closely related species is illustrated by the susceptibility of the ordinary sheep and the immunity of the Algerian variety to anthrax. Koch found that the bacilli of mouse septicæmia and the cocci that induce necrosis multiply simultaneously in the white mouse, but when field mice are inoculated with mixed cultures the latter infects while the former fails to develop. Even natural immunity is only relative and may be overcome (1) by massive doses of the virus as was demonstrated by Chaveau for Algerian sheep, (2) by lowering the temperature, as shown by Pasteur for chickens, (3) by starvation, as exemplified by Canalis and Morpurgo for pigeons—all with anthrax.

One very important thing that we have learned in recent years is that the ferments produced by the body-cells may be

and are modified under certain conditions. The cell may form a wholly new ferment, or one whose activity is so modified that it may be so regarded. It is either a new ferment or the old one greatly modified and intensified in its action. We have utilized this function of the body-cells for more than a hundred years in vaccination against smallpox, but this use has been wholly empirical until recently, when it was scientifically explained by the researches of von Pirquet. Smallpox virus is pathogenic to the man who has not suffered an attack of the disease or has not been vaccinated, while to the man who has recovered from the disease and to the one who has been properly vaccinated it is not pathogenic. By the introduction of the vaccine organism, which is a non-virulent form of the virus, the body-cells are trained, as it were, to digest and destroy its proteins, and this leads to the immediate destruction of the virus on subsequent exposure to the disease. The same principle holds in typhoid vaccination with the dead bacillus now so widely and successfully practised.

My students and I have convinced ourselves, at least, of the following: (*a*) The infective bacteria, taking the colon, typhoid, tubercle and the pneumococcus as types, contain an intracellular poison. (*b*) This is not a toxin because it is not destroyed by heat; it is not specific, it produces no antibody when injected into animals in increasing non-fatal doses. (*c*) These bacteria elaborate no soluble toxin or poison. In old cultures there may be a trace of poison but this results from the autolysis of the cells and is not a cellular secretion. (*d*) This poison can be obtained in soluble form only after cleavage of the cellular proteins, which may be accomplished by superheated steam, dilute acids or alkalies. (*e*) This poison is a group in the protein molecule. (*f*) It exists in all true proteins, in pathogenic and non-pathogenic bacteria and in vegetable and animal proteins. (*g*) It is a split product of the protein molecule. (*h*) It may result from the cleavage action of proteolytic ferments. (*i*) In most vegetable and animal proteins the poisonous group is neutralized by combination with non-poisonous groups; consequently such proteins have no poisonous action until they

undergo molecular disruption. (*j*) The poisonous group is common to all proteins; it is probably not chemically identical in different proteins, but is so nearly so that its gross toxicological action is the same. We designate it as the central or primary group in the protein molecule. (*k*) This primary group is poisonous because of the avidity with which it combines with secondary groups in the proteins of the animal body. (*l*) The specificity of proteins lies in their secondary, non-poisonous groups. It is in these that one protein chemically and biologically differs from another. (*m*) Biological relationship among proteins is determined by the chemical structure of their molecules. There are as many kinds of proteins as there are kinds of cells. (*n*) The specificity of the infective bacteria does not lie in the poisonous group of their proteins, for this has the same action in all, but in the non-poisonous groups. (*o*) The poison that kills in all the infectious diseases is the same. (*p*) The symptoms of the infections differ on account of the organ or tissue in which the virus accumulates and where it is split up and its poison liberated. (*q*) The ferment which causes the cleavage of the bacterial proteins in the different infectious diseases is specific. How strictly this is true can be determined only by more exhaustive and exact study.

When a fatal dose of a living virulent culture of the colon bacillus is injected into the peritoneal cavity of a guinea pig the following effects result: For a period of time which usually varies from eight to twelve hours the animal remains apparently normal. Its temperature may fluctuate slightly but not beyond the normal limits. The coat is not roughened and the position and behavior of the animal in no way distinguish it from its untreated fellows. This is the period of incubation and varies within certain time limits, but within these it is fairly constant. During this time the bacilli are multiplying enormously in the animal body. They are converting animal proteins into bacterial proteins. This is largely a synthetic or constructive process. The relatively simple, soluble proteins of the animal body with but little change are woven into the more complex structure of the bacterial proteins. The soluble

proteins of blood and lymph are built into the cellular proteins of the bacteria. There is no liberation of the protein poison and consequently no disturbance in the well-being of the host. It seems plain from this that the multiplication of bacteria in the animal body is not the direct and immediate cause of the symptoms of disease. When multiplication is most rapid and unobstructed there are no symptoms and in fact disease is not in evidence. During the period of incubation of an infectious disease the invading organism supplies the ferment; the soluble proteins of the animal body constitute the substrate; the process is constructive; simple proteins are built into more complex ones; no protein poison is liberated, and no recognizable symptoms mark the progress of the infection. Still, in the development of the phenomena of infection the period of incubation is critical, and the rate at which the infecting virus multiplies during this time is an important factor in determining the final outcome. The more virulent the virus, the more rapidly does it multiply and this means a larger amount of animal protein converted into bacterial protein. Rosenthal (Archiv. f. Hygiene, 1913, lxxxi, 81) has shown by means of his bacteriometer that the more virulent a bacterium the more rapidly does it multiply.

Somewhat abruptly there is a change in the behavior of our inoculated guinea pig. The hairs behind the ears begin to stand out and soon the entire coat becomes rough. It no longer eats, but retires to one corner of the cage and seems to be in distress. Slight pressure over the abdomen elicits evidence of pain and the temperature begins to fall and continues to do so until death. In case of recovery a rise in temperature is the first evidence of improvement. The characteristic lesion is a marked hemorrhagic peritonitis.

This somewhat abrupt change in the condition of the animal marks the end of the period of incubation and the beginning of the active disease. The animal cells have become sensitized and are now pouring out a specific ferment which digests the bacterial proteins. In the active stage of the disease, the animal cells supply the ferment; the bacterial proteins constitute

the substrate; complex cellular proteins are split into simpler bodies; the process is analytic and destructive; the protein poison is liberated; the symptoms of disease develop, and life is placed in jeopardy.

It must not be understood that the processes that characterize the period of incubation and those that develop the active stage of the disease are separated by a well-marked time limit and that the former wholly cease before the latter begin. This is not my understanding at all. Growth may be extending in one part of an organ, such as the lungs in pneumonia, while the destructive process predominates in another part. Only those cells with which the bacterial protein comes in contact are sensitized, and sensitization may be quite localized.

We take a second guinea pig and inject into its peritoneal cavity a fatal quantity of the dead cellular substance of the colon bacillus. In this experiment we cut ont one of the factors in the development of an infection, the growth of the bacillus in the animal body. This has been done *in vitro* and we inject into the peritoneal cavity enough of the cellular poison to kill. When this is done the animal remains quite well for about four hours, after which it shows symptoms identical with those manifested by its fellow which had been inoculated with the living culture. The lesions induced in both animals are the same. We conclude from this experiment that a period of about four hours is required to sensitize the cells of the guinea pig sufficiently to enable them to begin the cleavage of the bacterial protein and carry this process to the production of enough poison to so disturb the health of the animal that the effects come within the range of clinical observation. There are involved in the process of the incubation of an infectious disease two important functions. One is the growth of the invading organism and the other is the sensitization of the body-cells. The more rapid the growth of the virus and the greater the amount of foreign protein accumulated at the time when sensitization becomes effective the more disastrous are the results. Fortunately particulate proteins, like bacteria and protozoa, are not so effective in the production of sensitization as are the

simple, soluble proteins, such as those of blood-serum. Cell penetration is probably essential to the most perfect sensitization. Equally fortunate is it that the living cellular proteins are not so suddenly disrupted by the ferments produced by the body-cells as are the simple, soluble proteins.

To a third guinea pig we administer a fatal dose of the free protein poison split off from some protein molecule by either chemical agents or by a ferment acting *in vitro*. In this instance we cut out the whole period of incubation and the animal dies as quickly as it would from a dose of hydrocyanic acid. The infective agent has been grown artificially, the cleavage has been effected *in vitro* and the ready formed poison acts with the promptness that characterizes the action of other deadly chemical poisons. These experiments have been repeated in my laboratory many times with varied proteins, living and dead, particulate and in solution, of bacterial, vegetable and animal origin, and with their split products. If I have correctly interpreted them they throw much light on the phenomena of infection. However, before we question the correctness of the interpretation we must proceed with our experimentation.

The older literature shows that a few observers have long known that the parenteral introduction of diverse proteins is followed by the development of fever. There is an article by Gamaleia (Annales de l'Institut Pasteur, 1888, ii, 229), written twenty-five years ago, to which I wish to call attention. The title of the article is interesting: "The Destruction of Microbes in the Febrile Organism." Gamaleia showed that fever followed the parenteral introduction of dead as well as living bacteria, either pathogenic or non-pathogenic. He concluded from these experiments that fever is not a phenomenon of bacterial growth in the body. He found that the less virulent the infecting organism the higher and the more persistent is the fever. A rabbit inoculated with the anthrax bacillus runs a fever for only a few hours, when the temperature falls and death results; while one inoculated with the second vaccine runs a fever of three days. When a rabbit is inoculated with a

highly virulent anthrax bacillus, it may show but little or no elevation of temperature and dies within from five to seven hours. Gamaleia made similar experimental observations on other diseases and came to the following conclusion: The febrile process is not a result of the action of the bacteria, but on the contrary, is due to a reaction of the organism against their presence and results in their destruction. I feel that I am fully justified in offering these experiments, made a quarter of a century ago, as supporting my theory or explanation of the phenomena of infection.

In 1909 (Jour. Am. Med. Assn., Aug. 23) it was shown by work in my laboratory that fever could be induced experimentally in animals by the parenteral administration of proteins of diverse origin and structure, and that by modifying the size and frequency of the dose, the type of the fever could be determined at will. We produced an acute fever, the temperature rising to 107° and terminating fatally in a few hours, remittent and intermittent and continued fevers. The last mentioned furnished charts in no way distinguishable from those of typhoid fever. Not only can fever be induced but its accompaniments also. In continued fever thus produced there is increased nitrogen elimination, emaciation, loss of appetite, and lassitude and decreased urinary secretion. These experiments were amplified (Zeitschrift f. Immunitatsforschung, ix, 458) and have been confirmed by Friedberger and others. Protein fever, which includes practically all clinical fevers, is a result of parenteral protein digestion. In this process the animal cells supply the ferment and the foreign protein serves as substrate. The foreign protein may be living or dead, formed or without form. It may be detached or dead tissue from the animal's own body, as after burns. It may be absorbed from some mucous surface, as in hay fever. It may be artifically introduced, as in serum disease. It is usually a living protein, as in the infectious diseases.

There are two kinds of parenteral proteolytic ferments, nonspecific and specific. The former are normally present in the blood and tissues, especially in the first. They differ in kind

in different species and in amount and efficiency in different individuals. Their function is to digest and dispose of foreign proteins that find their way into the blood and tissues. Within limits they are general proteolytic ferments, as are those of the alimentary canal, but the variety of proteins upon which they act is more limited. They constitute the most important factor in racial and individual immunity. We are immune to most bacteria and protozoa, not because they do not elaborate poisons, for every protein molecule contains its poisonous group, but because they are destroyed by the general proteolytic enzymes as soon as they enter the body and are not allowed to multiply. These non-specific parenteral ferments are probably secretions of certain specialized cells, as the leucocytes. Under normal conditions these enzymes are capable of digesting those proteins upon which they act only in small amounts; but the cells which elaborate them may be stimulated in their activity. Whether or not these enzymes become specific when brought into contact with certain proteins has not been determined. The immunity secured by these enzymes is limited in extent and transitory in duration.

The specific, parenteral, proteolytic enzymes are not normal products of the body-cells, but are brought into existence under the stimulation of those proteins, introduced into the blood and tissues, which on account of their nature or amount escape the action of the non-specific ferments. A protein introduced into the blood and not promptly and fully digested by the non-specific enzymes is discharged from the blood current and deposited in some tissue, the cells of which after a time develop a specific ferment which splits up this protein while it is not capable of digesting any other. For certain proteins there are certain predilection organs and tissues in which they are stored, either exclusively or most abundantly: the pneumococcus in the lungs; the typhoid bacillus in the spleen, mesenteric and other glands; the viruses of the exanthematous diseases in the skin, etc.

For the development of the specific proteolytic ferments time is required and this varies with the sensitizing protein and

probably with the tissue in which it is deposited. The development of these ferments necessitates changes in the chemical constitution of the protein molecules of the body-cells and in this way the body-cells acquire a new function, which subsequently is brought into operation only by that protein to which its existence is due. As a result of this rearrangement in molecular structure the cell stores up a specific zymogen which is activated by contact with its specific protein.

Whether the products of digestion with the non-specific ferments and those elaborated by the specific enzymes are identical or not remains to be ascertained. The presence of a poisonous group in the protein molecules is disclosed in both enteral and parenteral digestion as well as by cleavage with chemical agents or enzymes *in vitro*. In enteral digestion the poison is most apparent in the peptone molecule, which is large, complex and non-diffusible. Further action of the alimentary enzymes splits the peptone into harmless amino-acids. The cleavage of proteins by chemical agents is a crude process, in which much of the poison is destroyed. When the poison is formed in the alimentary canal the animal is protected from its injurious effects by the walls and by its ultimate destruction. When the poison is liberated parenterally there are no protecting walls.

There are certainly other causes of fever, but the fever of the infectious diseases results from the parenteral digestion of the infecting agent by specific secretions elaborated by the body-cells; it is a phenomenon of the disposal of foreign and harmful material and it must be regarded as beneficent. However, there is a point above which it becomes a danger *per se*. In parenteral digestion the following sources of heat production must be evident: (1) The unaccustomed stimulation and consequent increased activity of the cells which supply the enzyme must be the source of no inconsiderable increase in heat production. (2) The cleavage of the foreign protein increases the liberation of heat. (3) The reaction between the product of the digestion and the tissues must lead to increased heat production. I regard the first and last of these as the more important sources of the over-production of heat in an infectious

disease. When the poison is liberated rapidly and abundantly the temperature falls and death is imminent.

There are many conditions which affect the course of a fever and some of these may be mentioned. Some viruses sensitize more quickly and thoroughly than others. It is probable that the living cells, so long as they are living, do not sensitize. Some of the virus protein must go into solution before cell penetration, which seems essential to thorough sensitization, can occur. A living colon bacillus of not more than twenty-four hours' growth, when injected intraperitoneally in a guinea pig, requires about ten hours to sensitize. With the dead bacillus the time is reduced to half, while with old autolysed cultures in which the sensitizing group is already in solution the time is still further shortened. Some pathogenic organisms, like the tubercle bacillus, have been so long parasitic that they have learned to protect themselves by deposits of fats and waxes. In this way they are probably protected to some extent against the destructive ferments elaborated by the body-cells. In all acute infections the destruction of the invading organism is modified and delayed by the altered relation between substrate and ferment and the accumulation of fermentative products. All these questions are but little understood and their solution must await further research.

I have given the new theory of the phenomena of infection as I understand it. The attack on these problems has only commenced and I do not hold that my opinions possess in every particular the force of demonstrated facts. If they prove to be provocative of further and more exact research I shall feel that they have been justified.

I shall now take up some of the facts for and against this theory and try to make impartial statements concerning them. In the first place it is true, as Doerr states, that it has not been conclusively demonstrated that the poison formed *in vitro* is identical with that elaborated *in vivo*. In fact we do not know the exact nature of the poison produced by the disruption of the protein molecule by chemical agents. I hold that this poison is a group in the protein molecule. Others question this and

hold to the endotoxin theory as first elaborated by R. Pfeiffer. So long as the poison was obtained only from complex proteins, such as bacterial cellular substances, the mixed proteins of blood-serum and egg-white which is known to be a protein mixture, my opponents had an argument which I could not meet, but some proteins, such as edestin, are believed by all students of protein chemistry to be chemical units, just as much so as crystallized bodies. We take edestin and split it into poisonous and non-poisonous portions. We inject the former into a fresh guinea pig and it kills the animal promptly after the development of certain definite and well-marked symptoms. We take another fresh guinea pig and sensitize it to edestin and after a proper interval we give the same animal a second injection of edestin. This animal develops the same symptoms in the same time and in the same sequence as the other and the postmortem findings in the two are identical. We know that the edestin injected into the animal contains the protein poison which we may liberate *in vitro*. It seems that the only conclusion justifiable from these facts is that in both instances the animal dies from the same poison and that by the process of sensitization the capability of splitting up the edestin molecules has been developed. Besides, the blood-serum of an animal sensitized to egg-white will, when incubated with egg-white in proper proportion *in vitro*, produce a poison which kills a fresh animal with the same symptoms and with the same postmortem condition as are developed on reinjection in a sensitized animal, while the blood of a fresh animal has no such action on egg-white *in vitro*. From these facts I draw the following conclusions: (1) The protein poison is a group in the protein molecule. (2) In a sensitized animal's blood-serum there is some agent capable of splitting up a protein and thus liberating a poison and that this something does not exist in the blood-serum of the unsensitized animal. If there be a fallacy in this reasoning I cannot see it. If it should be found that edestin and other proteins, believed to be chemical units, are not such, then my first conclusion is not wholly justified.

I do not claim that the protein poison formed *in vitro* by the

chemical disruption of the protein molecule is identical with that elaborated *in vivo* by specific ferments, but that they are closely related chemically is inferred from their physiological action. As I have stated, we do not know the chemical structure of the protein poison. We are certain that it is not an amino-acid, although it may be closely related to one of these. In its action the protein poison seems quite similar if not identical with the histamine of Barger and Dale. It will probably be found that the protein molecule contains a whole spectrum of poisons, one differing from another in some slight alteration in structure.

Years ago R. Pfeiffer demonstrated that cholera, typhoid, colon and many other bacilli secrete no toxin, but that the cellular proteins of these organisms are poisonous. In the abdominal cavities of animals previously treated with these bacteria, when new injections are made, the bacterial cells dissolve like sugar or salt in water, but notwithstanding this destruction of the bacteria the animal dies.

Indeed death is due to the destruction of the bacterial cells and the consequent liberation of the protein poison. When the amount of cellular substance is insufficient to furnish a fatal dose of the poison the animal survives and escapes infection. I regard Pfeiffer's phenomenon as the basis of lytic immunity and it must be evident that this form of immunity is not in any way comparable to that induced by toxins. Pfeiffer was certainly wrong in explaining this phenomenon on the supposition that the bacterial cell contains an endotoxin. The harmful contents of the cellular substance is not a toxin, in the sense that this term is now used, but is a poison. The next important work done along this line was that of Weichardt, who found that the blood-serum of rabbits previously treated repeatedly with placental proteins dissolves the same both *in vitro* and *in vivo* with the liberation of a poison. This experiment was a forerunner of Abherhalden's test for pregnancy. Next came the work of Friedemann, who showed that red blood-corpuscles may be dissolved without setting free an active poison, and, on the other hand, the poisonous group of the

hæmoglobin molecule may be extracted without dissolving the corpuscles. Thomsen demonstrated that in guinea pigs sensitized with erythrocytes there is no recognizable hæmolysis on reinjection, although anaphylactic shock results. When unbroken corpuscles are employed the anaphylactic poison may come from either the hæmoglobin or the stroma or from both. We have anaphylactized animals with hæmoglobin and with stroma. The former is easily done on account of the ready solubility of the hæmoglobin. The stroma is not so good an anaphylactogen for the opposite reason. Friedberger and Vallardi have found that only by having stroma, amboceptor and complement in proper proportions can the anaphylactic poison be prepared. Neufeld and Dold have found that anaphylactic poison can be prepared from bacteria without cytolysis. In regard to the anaphylatoxin of Friedberger it seems most likely that the matrix of this poison is the serum since it has been prepared by Bordet by incubating serum with agar and by Nathan by employing starch. Still, if it be proven that it comes from the serum, this in no way disturbs the theory that it is the protein poison. Our protein poison comes certainly from the protein molecule. It cannot be a ferment as we understand ferments at present. It is thermo-stabile and it elaborates no antibody and yet it may be identical with anaphylatoxin, for whether the latter comes from bacterial cells or from the serum it is of protein origin.

Loewitt and Barger (Archiv. f. exp. Path. u. Pharm., 1913, lxxiii, 164) have demonstrated that agar contains an anaphylactogenic protein. This does not prove that the poison produced in Bordet's experiments *does* come from the protein in the agar but it shows that this *may* be the source of the poison. The protein in agar undoubtedly has a large surface exposure and this renders it especially susceptible to ferment action. I have twice tried to prepare the protein poison by the cleavage of agar with chemical agents, but without success. However, this does not prove that a proteolytic ferment might not accomplish this purpose, since the chemical method is crude and destructive of a large amount of the poison.

Schlecht (Arch. f. exp. Path. u. Pharm., 1912, lxvii, 137) found that on reinjection of a sensitized animal the eosinophiles are increased, and Chancellor (Zeitschrift f. die gesamte exp. Med., 1913, ii, 29) finds that there is also an increase in the same corpuscles on the injection of the protein poison. So far as it goes this indicates that the poison liberated in anaphylactic shock and that formed from proteins by my method are similar in their effects.

The blood of an animal killed by anaphylactic shock coagulates slowly, while that of animals killed with the protein poison prepared by chemical agents coagulates in the usual time. This might be regarded as evidence that the poison formed *in vivo* and that prepared *in vitro* are not identical, but the agent which leads to retardation of coagulation may be one of the non-poisonous groups liberated on the cleavage of the protein molecules in the body. However, the protein poison obtained by chemical means from certain proteins, such as the tubercle bacillus, does prevent the coagulation of the blood. This is the first evidence that I have found of any dissimilarity in the action of the protein poison as obtained from different proteins. It has been shown by Loewitt (Arch. f. exp. Path. u. Pharm., 1912, lxviii, 85) and Waele (Zeitschrift f. Immunitätsforschung, 1913, xvii, 314) that a non-coagulable blood is not always in evidence in anaphylactic shock.

The fundamental fact in the work recently done by Abderhalden, the full import of which we cannot yet determine, rests upon the development of ferments as a result of the parenteral introduction of foreign proteins. How strictly specific these ferments are is a matter which must be measured by larger experience.

Weinland first showed that invertase is developed in dogs by the parenteral introduction of cane-sugar, and this work has been amplified by Abderhalden, Heilner and others until it has been demonstrated that the cells of the animal body can be trained to elaborate specific proteolytic, amylolytic and lipolytic ferments. The presence of specific ferments in the

blood-serum is now being used in the diagnosis of pregnancy, cancer and dementia præcox.

If I correctly interpret the recently reported experiments of Thiele and Embleton (Zeitschrift f. Immunitätsforschung, 1913, xix, 643, 666) they furnish strong evidence in favor of the theory which I have formulated. These investigators have developed the following points: (1) When the normal protective ferments of the animal body are inhibited in their activity, bacteria which under normal conditions are non-pathogenic become pathogenic. It is well known that ferment activity may be retarded by hypertonic saline solutions. When such non-pathogenic micro-organisms as *Sarcina lutea* and *B. prodigiosus* and others are suspended in from two to five per cent. salt solution and injected into the abdominal cavity of the guinea pig, the normal lytic ferment of the animal is inhibited; the micro-organism multiples and kills. In other words, a harmless bacterium is converted into a fatal one by holding in abeyance the normal protective function of the body. Years ago Buchner demonstrated that the alexin of blood-serum is highly sensitive to salt content, and by variations in this the activity of the ferment may be hastened, lowered or wholly arrested. In this connection it may be interesting to record the fact that some physicians believe that a heavily salted diet predisposes to pneumonia. (2) The blood and exudates of animals dying of infectious diseases are shown by the application of the ninhydrin and biuret tests to their diffusates to contain protein cleavage bodies, which are not present under normal conditions. These proteoclastic bodies could hardly have their origin elsewhere than in the cleavage of the bacterial proteins. (3) These cleavage bodies found in the blood of animals dying of the infectious diseases develop typical anaphylactic shock in fresh animals when injected intravenously.

The studies of the autolytic cleavage products, obtained from bacterial cellular substances, as reported by Rosenow (Jour. Infec. Dis., ix) and Cole (Jour. Exp. Med., xvi) show conclusively that the bacterial cells contain a poison—not an endotoxin, because no antibody can be produced. It is true that

these studies, taken alone, leave it in doubt whether the poison is a group in a larger molecule or constitutes a chemical entity, but surely the poison obtained from edestin and similar pure proteins must exist primarily as a group in a larger molecule. It must be admitted that the great weight of evidence is against the existence of endotoxins in the sense suggested some years ago by R. Pfeiffer.

Edmunds (Zeitschrift f. Immunitätsforschung, 1913, xvii, 105, also unpublished research) has shown that the physiological action of my protein poison in dogs and cats is essentially the same as that manifested in sensitized animals on reinjection and the same has been shown by several observers to be true in guinea pigs. While identity in physiological action does not establish chemical identity, it certainly suggests similarity in chemical structure.

Auer and Van Slyke (Jour. Exp. Med., 1913, xviii, 210), using the highly exact method of the latter for the determination of amino-nitrogen, find that the lungs of guinea pigs killed by anaphylactic shock on intravenous reinjection of from 0.5 to 0.9 c.c. of horse serum do not yield more amino-nitrogen than do the lungs of guinea pigs killed by injecting air into the veins directly after they have received intravenous injections of 0.9 c.c. of horse serum, and they conclude: "This investigation gives no support to the hypothesis that the true anaphylactic lung of the guinea pig is caused by protein split products." I am not inclined to attach much weight to this evidence, for the following reasons: (1) The total amount of protein introduced on reinjection was small; 0.5 c.c. of horse serum contains at the most not more than 40 mg. of total protein. (2) The lungs were weighed only to within 10 mg., *i.e.*, the error may have amounted to this. (3) The protein poison while still impure kills guinea pigs when given intravenously in doses of 0.5 mg. (4) While we do not know just what the protein poison is, we do know that it is not an amino-acid. (5) " In Table II when the free amino-nitrogen not only of the amino-acids but also of the peptones, albumoses, etc., was determined, we find the average after acute anaphylactic death

(ten animals) to be 61.8 mg. per 100 grammes of lung tissue, while controls (ten animals) show 58.5 mg. for the same amount of tissue; the small difference between the two averages (3 mg.) is without significance, as it falls within the range of normal variation." Three milligrammes of nitrogen represent at the least from fifteen to eighteen fatal doses of the protein poison. When a method for the recovery of a poison from the tissue fails to discover fifteen times the quantity necessary to kill, no great excellence for such a method can be claimed. (6) " When the non-coagulable amino-nitrogen after hydrolysis with hydrochloric acid was determined in five anaphylactic and five control lungs the results again showed no significant differences; the average yield of the anaphylactic lungs per 100 grammes of tissue was 172.6 mg., while the average for the controls was 171.2 mg." Here, there is in each 100 g. of the lungs of the anaphylactized animal enough more nitrogen, than in like tissue of the control, to account for from six to nine fatal doses of the poison. We know but little concerning the nature of the protein split products formed in parenteral digestion, but since they are recognized by the ninhydrin and biuret tests they cannot, wholly at least, consist of amino-acids. It is true that certain simple peptides may be split by parenteral ferments into their component amino-acids.

Acute anaphylactic shock is so striking in its manifestations that it has delayed studies of chronic protein intoxication, in which, I doubt not, there lies a rich and profitable field of research. Every foreign protein finding its way into the blood and tissues is more or less injurious to the body-cells. It may be directly harmful or it may act through its split products. When repeatedly introduced the body-cells become sensitized and split it up. When the intervals between the introductions are short there can be nothing like anaphylactic shock, but parenteral digestion has been established and the protein poison is liberated, possibly not in quantity sufficient to develop recognizable symptoms, but there results a chronic poisoning. If the theory which I have developed be true, the lesions of the infectious diseases are, in part at least, due to protein poisoning.

Moreover, the disease needs not be an infectious one in order to lead to either acute or chronic protein poisoning. The absorption of undigested or partially digested proteins from the alimentary canal may be quite as harmful as inoculation with a living virus. It seems to me that we are now quite justified in speaking of the "Albuminal Diseases," including under this title all health disturbances due to the parenteral introduction of foreign proteins, be they living or dead, organized or unorganized. The description given by Richet of his experiments on anaphylaxis in dogs suggests strikingly cholera nostras in man. Schittenhelm and Weichardt induced an "enteritis anaphylactica"; in like manner, Friedberger (Deutsche med. Wochenschrift, 1911, xxxvii, 481) developed pneumonia in sensitized guinea pigs by spraying horse serum into the trachea. This has been confirmed by Ishioka (Deutsche Arch. f. klin. Med., 1912, cvii, 500) and a careful histological study of the lungs in this condition has been made by Schlecht and Schwenker (ibid., cviii, 405). It seems highly probable that we have been wrong in believing that all diseased conditions are due to infections, many of which are secondary. In this connection I wish to call attention to the valuable research reported by Longcope (Jour. Exp. Med., 1913, xviii, 678), who has induced nephritis in rabbits and dogs by repeated injections of horse serum and egg-white. When we consider the care with which nature protects the body-cells from foreign proteins by the radical changes wrought in their structure by alimentary digestion, and since we know that every unbroken protein contains a highly poisonous group, we should proceed cautiously in the employment of serum and vaccine therapy. The value of diphtheria and some other antitoxins has been demonstrated and the good accomplished with these agents constitutes one of the great triumphs of modern medicine, but much of the protein therapy now so largely employed is without scientific justification. I have tried earnestly to so disrupt the protein molecule of certain pathogenic bacteria as to obtain a non-poisonous, sensitizing group which might be of value in either prophylactic or curative treatment, but without practi-

cal success. I have obtained from the cellular substances of the colon, typhoid and tubercle bacilli non-poisonous sensitizing proteins. Those from the colon and typhoid give some degree of immunity to subsequent inoculation with respective living cultures, but the protection thus secured is low in degree and ephemeral; while that from the tubercle bacillus fails to protect experimental animals. I must therefore report failure with glimpses of that will-o'-the-wisp which haunts the laboratory of every investigator. When one thinks of the great number of cleavage lines that run through the large protein molecule he must not be surprised when the gem with perfect facets, which he seeks, is not revealed at the first stroke of the hammer.

As has been stated, we have split proteins into poisonous and non-poisonous portions. This has been done with proteins of most diverse origin, bacterial, vegetable and animal, and we have found no true protein which has failed to undergo this cleavage. Certain pseudo-proteins, like gelatin, do not respond to this test, but all true proteins, as far as tested, have been split into poisonous and non-poisonous portions. This is the foundation stone of our theory of protein sensitization. All true proteins are sensitizers, and so far it has not been shown that sensitization can be established by any non-protein substance. All sensitizers develop symptoms of poisoning on reinjection. These symptoms induced by reinjection are identical in manifestation and sequence with those induced in the fresh animal by the injection of the poison split off from the protein molecule by chemical agents, or by the ferments in the serum or organ extracts of sensitized animals. Therefore, we have concluded that anaphylactic shock is due to the cleavage of the molecule of the protein sensitizer on reinjection, and the liberation of the protein poison, and this cleavage is due to a specific proteolytic enzyme developed in the cells of the animal body as a result of the first injection. We have repeatedly shown that the poisonous group obtained from the protein molecule by cleavage with chemicals or with ferments does not sensitize animals. This is contrary to the generally

accepted view, and our claim on this point has met with either silence or denial, but we have tested this matter so often and with poisons obtained from so many and such a variety of proteins that we have no hesitancy in affirming that the poisonous group in the protein molecule does not sensitize animals. But it is said that toxins are necessary to elaborate antitoxins, and that the latter can be produced in no other way. This is true, but the protein poisons are not toxins, and they lead to the elaboration of no antibodies. The toxins are specific; the protein poisons are not. The blood-serum of an animal treated properly with a toxin neutralizes the toxin both *in vitro* and *in vivo,* while the blood-serum of a sensitized animal renders the protein with which the animal has been treated, when brought in contact with it under proper conditions, either *in vitro* or *in vivo,* poisonous. It seems to us that it has been positively demonstrated that the sensitizing and toxic groups in the protein molecule are not the same. It might be argued that in ordinary protein mixtures, such as blood-serum and egg-white, one protein may contain the sensitizing group and another the toxic group. This may be true, but when pure proteins, such as edestin, are used the two groups must exist in the same molecule. The specificity of proteins is demonstrated in sensitization. The toxic group shows no specificity. This property characterizes the sensitizing group, and it is in these groups that the fundamental and characteristic property of each protein resides. The exact structure and chemical nature of neither the sensitizing nor the poisonous groups have been determined. The latter seems to be physiologically the same in all proteins, the former is specific in every protein. By our method, the poisonous group is easily obtained; not in a chemically pure condition, but so that its presence can be demonstrated. The poisonous group, being the same in all proteins, is obtained from all by the same or by like methods. The sensitizing group, being the same in no two proteins, cannot be isolated from all by the same method. We have been able to obtain specific sensitizing groups from colon, typhoid and tubercle protein quite uniformly. From the pneumococcus

and related organisms we have never succeeded in obtaining a sensitizing group. From egg-white we have rarely succeeded, generally failed. It seems evident to us that the sensitizing groups in many proteins are highly labile bodies, probably of such delicate structure that they easily fall to pieces.

If sensitizers are ever to have a legitimate place in the treatment of disease, it will be of the highest importance to obtain them free from the poisonous group. Every time an unbroken protein is introduced into the body it carries with it, and as a part of it, a poison. From the very careless, rash and unwarranted way in which "vaccines" of most diverse origin and composition are now used in the treatment of disease, this matter certainly cannot be understood or its danger appreciated by those who subject their patients to such risks. It should be clearly understood that all proteins contain a poisonous group—a substance which in a dose of 0.5 mg. injected intravenously kills a guinea pig. This poison is present in all the so-called "vaccines" now so largely used, and it is not strange that death occasionally follows the use of "phylacogen" or similar preparations. Not only do these proteins contain a poison, but when introduced parenterally the poison is set free, not in the stomach, from which it may be removed, but in the blood and tissues. It is possible that vaccine therapy may become of great service in the treatment of disease. Even now there are occasional brilliant results which are reported while the failures and disasters are not so widely advertised. But before sensitization can be of great service in a therapeutical way we must secure sensitizers free from poisonous constituents. Until recently the existence of, or the possibility of preparing non-toxic sensitizers has been made evident only by our work. Recently, confirmations of our studies along this line have come: (1) From White and Avery (Jour. Med. Research, 1912, xxvi, 317), who have prepared by our method a sensitizing group from tubercle cell substance. (2) From Zunz (Zeitschrift f. Immunitätsforschung, 1913, xvi, 580), who, as the result of a most exhaustive research, has shown that one

of the primary albumoses (the synalbumose of Pick) sensitizes, but does not induce anaphylactic shock on reinjection. Zunz states: Both active and passive anaphylaxis can be induced by the three so-called primary proteoses (hetero-, proto-, and synalbumose), but not by thioalbumose, nor the other so-called secondary proteoses, nor by Siegfried's pepsin-fibrin-peptone-β, nor by any of the abiuret products of peptic, tryptic, or ereptic digestion.

Animals sensitized with hetero-, proto-, or synalbumose develop anaphylactic shock on reinjection with the original serum, acid albumin, hetero- or proto-albumose, but *not* after reinjection with synalbumose, thio-albumose, the other secondary proteoses, pepsin-fibrin-peptone-β, or any of the abiuret products of peptic, tryptic, or ereptic digestion. The hetero- and proto-albumoses both sensitize and induce anaphylactic shock, while synalbumose sensitizes only. It follows, therefore, that sensitization and the production of anaphylactic shock are due to different groups in the protein molecule.

Wells and Osborne (Jour. Infect. Dis., 1913, xii, 341), working with the purest vegetable proteins known, hordein from barley, glutinin from wheat, and gliadin from both wheat and rye, find that:

> Guinea pigs sensitized with gliadin from wheat or rye give strong anaphylactic reactions with hordein from barley, but these are not so strong as the reactions obtained with the homologous protein. Similar results are obtained if the sensitizing protein is hordein, and the second injection is gliadin. We have here a common anaphylaxis reaction developed by two chemically distinct but similar proteins of different biological origin, thus indicating that the specificity of the reaction is determined by the chemical constitution of the protein rather than by its biological origin. This is in harmony with the fact that chemically closely related proteins have, as yet, been found only in tissues biologically nearly related.
>
> From the results of these experiments it seems probable that the entire protein molecule is not involved in the specific character of the anaphylaxis reaction, but this is developed by certain groups contained therein, and that one and the same protein molecule may contain two or more such groups.

Evidently the view that the protein molecule contains a sensitizing group, one or more, is finding strong experimental support. In our opinion this view was demonstrated by Vaughan and Wheeler (Jour. Infect. Dis., iv, 476) as early as 1907, but recent work, such as that by Zunz, Gay, Wells and Osborne, and others, strengthens the evidence then offered. According to our theory, every protein molecule contains a chemical nucleus, keystone or archon. This is the protein poison, and is physiologically much the same in all proteins. One protein differs from another in its secondary or tertiary groups. In these resides the biological specificity of proteins. Biologically related proteins contain chemically related groups, and in these are found the sensitizing agents. The chemical structure of the protein molecule determines its biological differentiation and development. It is not, therefore, surprising to find that a pure protein from wheat sensitizes to another closely related protein from such a biologically closely related grain as rye. This, however, does not indicate that the proteins from the two grains are wholly identical in chemical structure. It only shows that the two protein molecules contain among their secondary groups identical or closely related atomic combinations. The same can be said of the fact that certain non-pathogenic acid-fast bacteria may, at least partially, sensitize animals to the tubercle bacilus. Biological relationship is determined by the chemical structure of the protein molecule. We hold this to be true of all specific biological tests for proteins, whether they be agglutination, precipitin, lytic, complement deviation, or anaphylactic tests. The chemical structure of the protein molecule determines all these. The form and function of every cell is determined by the chemical structure of its constituent proteins. That the sensitizing agent in the protein molecule resides in its secondary groups is shown by: (*a*) the fact that sensitization is within limits specific; (*b*) the fact that the residues left after stripping off these secondary groups by proteolytic digestion or by the action of dilute bases and acids, do not sensitize. Peptones, polypeptides, amino-acids, and

the protein poison do not sensitize to either themselves or to the unbroken proteins from which they have been derived.

We regard the work of Jobling and Bull (Jour. Exp. Med., 1913, xvii, 453) as confirmatory of our studies in every particular. These investigators have studied the action of the cellular substance of the typhoid bacillus and its split products, produced by the action of a proteolytic ferment obtained from leucocytes, and state their findings as follows:

> Freshly washed, unheated typhoid bacilli intravenously injected into dogs cause the development of definite symptoms as early as twenty minutes after the injection. Boiling for ten minutes does not destroy the toxic effects of a freshly washed bacterial emulsion. Complete solution of the bacteria (in dilute alkali) of a fresh emulsion does not prevent the removal of the toxic substance with the coagulable proteins. The action of leucoprotease splits the toxic substance to a non-coagulable state, the digested mixtures being toxic after removing the coagulable portion. The mere presence of the leucocytic ferment is not responsible for the toxicity of the filtrate from the digested mixture, and continued digestion destroys the toxicity of a previous toxic mixture. From these observations it is concluded that the toxic properties of freshly washed typhoid bacteria are not entirely due to preformed secretory toxic bodies that are stored in the bacterial bodies, but that these properties are due largely to products formed by hydration of the bacterial proteins through the agency of ferments present in the circulation of the animal previous to the injection, or which become mobile subsequent to the entrance of the foreign bodies into the blood-stream. Since leucocytic ferments can attack the bacterial proteins *in vitro*, it is possible that the leucocytes are a source of the ferments which are active in experimental and natural cases of intoxication with the whole bacteria.

The studies of Pick and Obermeyer (Wiener klin. Wochenschrift, 1904, 1906 and 1912), confirmed and amplified by Landsteimer and Prasek (Zeitschrift f. Immunitätsforschung, 1913, xx, 211), render it highly probable that the specificity of the protein molecule is closely connected with its aromatic group. Furthermore, it is worthy of note that gelatin, in which the aromatic group is wanting, is not a sensitizer. The above-mentioned investigators have shown that when certain substitutions are made in the aromatic group of a protein it loses its specificity. It is also noteworthy that gelatin does not yield

the protein poison when disrupted by our chemical method. It seems, therefore, that gelatin contains neither the sensitizing nor the poisonous group.

I must protest against classifying the toxins and anaphylactogens together under the name of "antigen." This term should be reserved for the former. The anaphylactogens produce no antibody. Pick (Kolle and Wassermann Handbuch, zweite Auflage, i, 698) very properly states that diphtheria toxin elaborates neither a precipitin, agglutinin, nor hæmolysin and that it is not an anaphylactogen. He proposes that the toxins be designated as monovalent antigens in contradistinction to the polyvalent antigens which elaborate numerous immune bodies. I can see no reason for calling the anaphylactogens antigens. The anaphylactogens are colloids of highly complex molecular structure, while the latest research all points to the non-protein character of the toxin. As Pick states: Faust finds the active principle of cobra and crotalus venom to be a nitrogen-free sapotoxin; Abel and Ford report that the hæmotoxin of *Amanita phalloides* is a glucoside containing nitrogen and sulphur; Bang and Forssmann state that the hæmolytic component of the red corpuscle is a lipoid; Jacoby claims to have obtained a nitrogen-free ricin; and according to Burckhardt the hæmolysin of *Bacterium putidum* is non-protein. Likewise, tuberculin is an anaphylactogen or not according to the preparation. When free from other constituents of tuberculo-protein it is neither anaphylactogen nor toxin, but a poison. Tuberculo-protein contains an anaphylactogen group but this does not constitute the active principle of tuberculin, which is of relatively simple structure.

Many investigators have failed to sensitize animals with tuberculin, while most have succeeded with dead bacilli and with aqueous extracts. This is not surprising; indeed it is what should have been expected. Tuberculin consists of digested, denatured proteins of relatively simple composition. It is well known that peptones and polypeptides do not sensitize. The protein poison when detached from other groups in the protein molecule sensitizes neither to itself nor to the un-

broken protein. The fact that tuberculin does not sensitize or does so imperfectly raises a serious question as to its employment as a therapeutic agent. It is undoubtedly an excellent diagnostic agent because its relatively simple structure may favor its prompt cleavage when injected into an animal already sensitized by the disease. But if it is not a sensitizer its therapeutic good effect, if it has any such effect, must be confined to the possible establishment of a tolerance to the tuberculo-poison. Sensitization to tuberculo-protein can be induced by bacillary emulsions, with watery extracts, and with the non-poisonous residue. If the sensitization secured by the last-mentioned agent is as good as that produced by the others, it has the advantage of not containing any poison. On the other hand, if the therapeutic effect desired consists in the development of a tolerance to the poison, tuberculin must be preferred unless we should use the more completely isolated poison.

There is one statement in the criticism of Doerr, quoted in the beginning of this lecture, to which I have not as yet referred, and which is of great interest. This is the well-established fact that during an attack of measles the person cannot be sensitized to tuberculin. I shall not attempt to explain this phenomenon, but I wish to emphasize its importance. If the theory which I have attempted to develop be true, we have more or less immunity, inherited or acquired, to tuberculosis. This is due to the fact that the cells of our bodies supply ferments more or less destructive to the *Bacillus tuberculosis.* Through inheritance or through previous exposure to this infection, this slight immunity or increased resistance has been developed. In measles it disappears or at least is held in abeyance. Why, we do not know, but it is interesting to call to mind how many cases of tuberculosis begin to develop in an attack of measles. The ubiquitous *B. tuberculosis* strikes when the shield is down.

COLLOIDAL REACTIONS AND THEIR RELATIONS TO BIOLOGY*

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IF one dissolves cane-sugar in water, the sugar exists in the solution in the form of molecules. These molecules are so small that they cannot be seen even with the very best microscope and they cannot be separated from the solvent, even with the very finest filter. In addition to this there exists a very powerful attraction between the water and the dissolved molecules, in consequence of which the dissolved substance cannot in any way be separated from the solvent except at the expense of a considerable amount of work in some form. This attraction between the dissolved substance and the solvent was first measured by Pfeffer for cane-sugar, and from his results Van't Hoff calculated that this power equals the pressure of a gas of the same molecular concentration and of the same temperature as the solution itself. Therefore it is very often assumed that the particles of a dissolved substance exercise a pressure against the surface of the fluid in which it is contained. This pressure has been given the name of *the osmotic pressure.*

The experiments of Pfeffer have been recently repeated by American investigators, namely Morse, Frazer and their collaborators, and their results, which have been obtained by the aid of very elaborate apparatus, fully confirm the results of Pfeffer and the conclusions of Van't Hoff so far as the solutions of cane-sugar and of grape-sugar are concerned.

There are various methods of determining osmotic pressure, of which the most usual ones are the determinations of the boiling point and of the freezing point of solutions. If the

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osmotic pressure represents an attractive force between the dissolved substance and the pure solvent, then this pressure must offer a certain resistance to the separation of both. The separation of the solvent from the dissolved substance is effected when the solvent is boiled away, and when it is frozen out in the form of ice. Therefore, the boiling point of a solution of a non-volatile substance is higher than the boiling point of the solvent and the freezing point of a solution is lower than that of the pure solvent. On the other hand, solutions of the same osmotic pressure show the same boiling point and the same freezing point; and this, consequently, must be the case with solutions of different substances that, in the same volume, contain the same number of molecules. One molecule, therefore, exercises the same influence upon elevation of the boiling point or upon depression of the freezing point, quite independently of the size of the molecule, whether it is large or small, heavy or light. From this it follows, that equal weights of different substances, when dissolved in water to the same volume, show different osmotic pressures; as a matter of fact, the one with the lower molecular weight prevails as far as the elevation of the boiling point or the depression of the freezing point is concerned. Therefore, if a pretty strong solution shows the same or nearly the same boiling point and freezing point as the pure solvent, one must conclude that the particles of the substance in solution are very large in comparison with common molecules. There is a whole class of substances that behave, in watery solution, in the way just mentioned, namely the colloids. Therefore, these substances must exist in solution either in the form of very large molecules or as aggregations of molecules.

It is well known that Graham classified substances into two classes: crystalloids and colloids. The distinction between the two classes depended upon the different sizes of the particles in solution, not upon chemical differences. The difference being of a physical kind, a given substance, under different conditions, may occur in both forms. This is the case with soaps, which in alcoholic solution behave like crystalloids; in watery solution, like colloids. I have already pointed out that the colloids show

hardly any osmotic pressure. Furthermore, some colloidal particles can be seen through the ultramicroscope, and can be separated from the solvent by filtering through filters that have been "tightened" by such a substance as collodion or gelatin. In this way blood-serum can be freed from proteins so that it does not give precipitate with tannic acid. The difference between crystalloids and colloids, which was first noted by Graham, was that crystalloids readily diffused through membranes, whereas colloids did not.

Colloids are very important in physiological chemistry. One might even say that the living organism is mainly built up of colloids. To this group of substances belong the proteins that form the main part of the animal body, and the complex carbohydrates that occur in the plants and form their main constituents.

Colloidal substances may enter into reactions with each other and with crystalloids; and different particles of the same colloid may combine with each other. The most usual way in which colloidal reactions are observed depends upon visible precipitation; but it is easily conceived that reactions might take place in a solution without producing a visible precipitate. I shall be able to point out several reactions of this kind, in which colloïdal substances take a part.

Among the colloidal substances in the living organisms, the so-called enzymes are very important, since they are responsible for many of the processes in the organism. Possibly enzymes are not typical colloids, and perhaps, in pure form, they may not be classified as such; but if they are not colloids, they certainly behave like colloids.

Between certain colloidal solutions and true, fine powders suspended in water, there is no sharp distinction. As a matter of fact, there is some agreement between them in the way in which they are thrown from solutions or suspension. From the behavior of suspended powders towards other substances, useful knowledge has been obtained regarding the properties of true colloids. The reactions of powders are more readily studied than those of colloids, because powders can be easily

removed from fluids whilst it is very difficult to separate certain colloids from their solvents.

It is a known fact, that fine powders, *e.g.*, charcoal, possess the faculty of taking up certain substances dissolved in water and of attaching them to their surfaces. This process has been called *adsorption* and is to be distinguished from the process called *absorption,* which occurs when a substance is dissolved by a *fluid.* If a substance is divided between two solvents, that do not mix with each other, it is absorbed into each of them; and the final proportion between the concentrations of the substance in the two solvents is constant, independent of the total amounts dissolved.

On the other hand, if a solid powder, say charcoal, is shaken up in a watery solution of, say, an organic acid, then part of the acid is taken up by the charcoal, but the amount taken up never bears a constant relation to the amount left behind in solution. And if the amount of charcoal and of fluid remains constant, then the charcoal takes up *relatively* more from a dilute solution than it does from a concentrated one; and beyond a certain limit, nothing more is adsorbed, if the amount of substance in solution is increased. But, of course, the absolute amount of substance adsorbed is larger, up to a certain limit, the greater the concentration in solution.

The adsorption of a crystalloid substance by means of a powder is a reversible process. Therefore, the substance adsorbed can be partly removed from the powder by adding water. The final equilibrium in the distribution of the substance between the solution and the powder is the same, independent of the way in which it is attained, whether all the substance was adsorbed by the charcoal, or present in the solution, to start with.

Colloidal substances are also adsorbed by powders. For some cases of adsorption of colloids the same curve has been obtained as for the adsorption of crystalloids. By using a sufficient amount of adsorbing powder, the whole amount of colloid can be removed from solution. In this way the pro-

teins of blood-serum can be completely removed with such powders as kaolin.

An important case of adsorption of colloids is represented by the adsorption of enzymes. It has long been known that enzymes are taken up by powders or by precipitates. Some years ago I conducted quantitative investigations with regard to this process. The enzymes employed in my experiments were trypsin (the proteolytic enzyme of the pancreatic gland) and rennin, with charcoal as the chief adsorbing substance.

It was found, in the first place, that the amount of enzyme taken up by the charcoal depends very much upon the time of interaction between the charcoal and the enzyme, as well as upon the temperature at which the interaction takes place, up to a certain limit more enzyme being adsorbed the longer the time and the higher the temperature. Furthermore, the amount of enzyme finally taken up is independent of the amount of water present, which is, I think, an important observation, since it shows that the adsorption process in this case is not reversible, the water not being capable of removing anything of the adsorbed enzyme from the charcoal. The fact that the enzyme once adsorbed by the charcoal cannot be set free again by the addition of water indicates, I think, a very important difference between the adsorption of enzymes and the adsorption of crystalloids. The enzymes are in fact in some way attached to the charcoal. Only when another substance is added that, like the enzyme itself, is adsorbed by the charcoal, can a small part of the enzymes be set free again. In this way trypsin adsorbed by charcoal can be liberated, to a slight extent, by casein; and rennin, adsorbed by charcoal, can be partly set free again by the action of egg-albumin, by some constituents of milk, and by saponin. For the same reason these substances are capable of diminishing the adsorption of the same enzymes by charcoal, if they are present when the adsorption takes place, their effects being much more pronounced under such conditions than when they are added after adsorption is complete.

Very often the substrate of an enzyme (the substrate of an enzyme is the kind of substance upon which the enzyme is

capable of acting), when acting upon charcoal charged with enzyme, sets part of the latter free. This effect of substrate is more marked if it is added when adsorption is in progress than if it is added after completion of the process. For this reason the order in which the three interacting substances—namely, charcoal, enzyme and substrate—are mixed with each other is very important so far as amount of free and active enzyme is concerned, more enzyme being left free if the substances are mixed in the order charcoal, substrate, enzyme, than if the substrate is added after the adsorption of enzyme is complete. This observation depends, of course, upon the fact that the compound charcoal-enzyme once formed is very stable and is not reversible in the usual sense of the word.

When an enzyme is adsorbed by a powder, it loses its power of acting upon the substrate, unless the substrate is capable of liberating part of the enzymes, in which case the part set free becomes active again. This fact supports the general view that an enzyme previous to its action must combine with substrate in some way, and that no enzymation takes place unless this combination is effected. If another substance is present that, like the substrate, is capable of combining with the enzyme, then the amount of enzyme in union with the other substance is put out of action.

Several substances other than powders are capable of inhibiting the actions of enzymes. Thus the action of trypsin is checked by normal blood-serum and rennin is inhibited in its activity by normal serum as well as by egg-white. I have had an opportunity of studying the inhibiting processes referred to, and have found that the inhibiting action of normal serum upon trypsin and upon rennin is influenced by the same factors as that of charcoal. If the inhibiting action is studied in neutral solutions, the influences of time and temperature are uniform, and the amount of water present during the interaction of the serum and the enzyme is of no importance at all. The non-influence of water upon the final result of the reaction indicates that the reaction takes place between substances that are not really dissolved in water—between colloidal particles in the

serum and the enzyme. Therefore, the reaction might be classified as a colloidal reaction, having much in common with the adsorption of enzymes by charcoal.

I have already pointed out that when charcoal, charged with trypsin, is acted upon by casein, a small part of the enzyme is set free. In this case the amount of water present during the action of the casein is without influence upon the amount of enzyme set free. Therefore, the reaction must take place between particles not really dissolved in water, that is to say, between colloidal or solid particles. The influences of time and temperature are likewise the same as when trypsin is adsorbed by charcoal. Therefore, I believe that some of the enzyme is set free because some of the casein is adsorbed by the charcoal. Since all substances capable of liberating enzymes from enzyme-charcoal combinations are adsorbed by the latter, I believe that charcoal, charged with enzyme, adsorbs some of the added substance and therefore, in the process alluded to above, has to give off some of the enzyme previously adsorbed—there would be no room for the whole amount of the enzyme and the new adsorbable substance, at the same time, upon the surface of the charcoal.

Jahnson-Blohm found that some colloids are capable of setting free some of the rennin that had previously been rendered inactive by blood-serum. Even in this instance, time and temperature play the part they do in the case of charcoal, indicating that the two processes are of the same kind.

I have already pointed out that according to the view of many investigators the enzymes combine with the substrate and that no enzymation takes place unless this combination is effected. Of what nature is this combination? I am inclined to think that it must be dissimilar in different cases, according to the various properties of substrate, and perhaps also of enzyme. Some substrates are colloids, *e.g.*, proteins, and others, *e.g.*, cane-sugar, are crystalloids. When the substrate is a colloid, its compound with the enzyme is very likely to behave in some respects like the compounds between two colloids, already referred to. If only part of the enzyme combined with the sub-

strate is active, then the effect of the enzyme (in a short time) ought to be proportional to the amount of enzyme combined. When trypsin acts upon albumin in a perfectly neutral solution, the effect is independent of the amount of water present; consequently, the amount of enzyme combined with the substrate is independent of the amount of water. This is the observation that has been made before, with regard to combinations between two colloids, namely, enzymes and inhibiting substances. In the latter case the enzyme was attached to the inhibiting substance. No attachment like that occurs between the enzyme and the substrate, for the simple reason, I believe, that the substrate is broken up by the action of the enzyme; the enzyme, therefore, is set free and can combine with another part of the substrate.

When the substrate is a crystalloid, its combination with enzyme seems to depend very much upon the amount of water present, that is to say, upon the degree of concentration of the substrate. Thus, when cane-sugar is split up by means of invertin, not only the cane-sugar, but also its cleavage products, combine with the enzyme, a fact that makes the study of the action of invertin somewhat complicated. However, if one works with sufficiently dilute solutions, the action of the products is eliminated. If one estimates in this way the amount of cane-sugar taken up by a given amount of enzyme from different amounts of sugar added, one obtains a curve like that representing the adsorption of a crystalloid substance by a powder.

The inhibiting action of normal serum does not show any specific properties, that is to say, the serum inhibits the action of a certain kind of enzyme in the same way, and to the same extent, independently of the species of animal from which the enzyme is obtained. In this connection I should like to call your attention to some experiments I have made which seem to indicate that substances of a specific inhibitory power may be normally present in the animal body. These experiments refer to the form in which rennin is present in the body.

According to recent investigations of Hammarsten, rennin is a proteolytic enzyme, its best known property being its power

of clotting milk. It is formed in the mucous membrane of the stomach, where it seems to be present in an inactive form. Hammarsten, some forty years ago, found that a neutral infusion of the mucous membrane of the stomach increases very much in activity if it is treated with very dilute acid solution and then is neutralized. On the other hand, the inhibiting substance present in normal serum is destroyed when it is acted upon by acid. Therefore, an inactive combination between rennin and serum likewise acquires activity if it is treated with dilute hydrochloric acid. The same is the case with the inactive combination between rennin and some inhibiting substance in the white of egg. The combinations between rennin and the inhibiting substances in the serum and in the white of egg behave against dilute acids, therefore, like the so-called zymogen of the rennin (the form in which the rennin is present in the neutral infusion of the mucous membrane of the stomach). In both cases activation takes place under the influence of the acid; in the former case, in the combinations between the rennin and an inhibiting substance.

The activation being effected by the destruction of the inhibiting substance, I tried to find out whether the zymogen of the rennin is a combination between the rennin and some inhibiting substance, that might be destroyed by acid. If this view were right then it would perhaps be possible to prepare in some way, from the zymogen, an inhibiting substance. I tried very long in vain to do this, but finally I succeeded in the following way. The zymogen had to be prepared without the reaction turning acid.

The mucous membrane of a calf stomach was infused with water (1:15) and some calcium carbonate was added. After 24 hours at a low temperature the mixture was filtered, the filtrate was diluted with 3 volumes of water and treated for some 30 minutes at 37° C., with a very weak solution of ammonium hydroxide. The fluid was then neutralized and its influence upon calf rennin determined. Before treatment with ammonia, the infusion contained some free rennin, but after the treatment it inhibited the action of added rennin. When the

zymogen, after the action of ammonia, was treated with very dilute hydrochloric acid solution and neutralized, the presence of free rennin could again be shown. When the solution was treated once more with ammonia, the inhibiting substance could not be obtained.

In my opinion the zymogen is a combination between rennin and an inhibiting substance, both of them being formed in the mucous membrane of the stomach. In a neutral infusion rennin must be present in some excess, because the infusion clots milk. When the zymogen is acted upon by an acid, all or at all events the greatest part of the inhibiting substance is destroyed, and in that way all, or nearly all, of the rennin is set free. If on the contrary the zymogen is treated with weak ammonia, some of the rennin is destroyed and consequently some of the inhibiting substance is set free and checks the action of added rennin. But the main part of the inhibiting substance remains in combination with rennin; and, therefore, the inhibiting solution treated with hydrochloric acid again clots milk. It seems that rennin and the inhibiting substance, when combined, are able to protect each other up to a certain degree against deleterious influences, and therefore not all of the rennin in the zymogen can be destroyed by ammonia; the main part of the same remains combined with the checking substance. The destruction of the inhibiting substance by means of an acid seems to be much more complete.

If my view on the nature of the zymogen of the rennin is right—and nothing is known to prove that it is not—this zymogen is an instance of an enzyme whose action is *normally* controlled by an inhibiting substance, both being formed in the mucous membrane of the stomach.

When the inhibiting substance is prepared from the zymogen by treatment with ammonia, the concentrations of zymogen and ammonia are very important. If the proper concentrations found by experiment are not observed, then the inhibiting substance may not be obtained. All rennin-zymogens obtained from different kinds of animals are activated by acids and therefore seem to consist of rennin and inhibiting substance; but in a few

cases only have I succeeded in obtaining, by treatment with ammonia, an inhibiting solution. I have been successful only with zymogens of calf, guinea pig and pike rennins. The inhibiting substances obtained from these sources easily or principally inhibit the action of rennins from the corresponding species of animal, that is to say, the inhibiting action is specific. This is the very first instance in which a specific inhibiting action has been observed without immunization. I am going to show some figures, from which the specificity of the inhibiting action can be seen.

In my experiments the inhibiting action was specific with one very marked exception: the checking substance, obtained from calf stomach, inhibited the action of sheep rennin as well as that of calf rennin. I rather think that this might depend upon a close relation of these species to each other, both of them being ruminating types of animals. Since it had not been determined whether antibody, obtained by immunizing with calf rennin, inhibits the action of sheep rennin as well as that of calf rennin, I immunized rabbits against calf rennin in order to ascertain whether this is the case. The immunization was readily effected and was found to pertain to both kinds of rennin, although upon sheep rennin perhaps to a less extent than upon calf rennin. None of the other kinds of rennin tried was influenced by the immune serum obtained.

With regard to different kinds of rennin, therefore, the immune serum obtained with calf rennin and the inhibiting substance prepared from calf stomach behave in the same way. The conformity between the two substances goes further than that. Both are destroyed by very slight proportions of hydrochloric acid, even when they are combined with calf rennin, in which case, therefore, the rennin is set free and becomes active. The two substances agree, also, in the fact that calf rennin combined with them is partly destroyed by weak ammonia, the inhibiting substance being, therefore, partly activated. If the fluid so obtained is treated with hydrochloric acid, the checking substances are destroyed and the rennin is activated. Although the two inhibiting substances agree, therefore,

in several respects, it seems rather doubtful that they are identical, since the antibody obtained by immunization loses its activity on being heated to 100° C., or before that temperature is attained, but the inhibiting substance obtained from the mucous membrane of the stomach is not completely destroyed at 100° C.

The most important features of the colloidal reactions I have been speaking of are the following:

1. They do not take place in constant proportions.

2. The reaction between one colloid and one crystalloid depends very much upon the concentration of the fluid, but the reaction between two colloids does not. In the latter case, the two substances are sometimes attached to each other in such a way that only by the action of a third substance can they be partly set free again.

3. The formation of a compound between an enzyme and its substrate seems to be a condition necessary for the activity of the enzyme. Furthermore, other substances than the substrates are capable of combining with the enzyme. These substances may prevent the combination between enzyme and substrate, and may thus inhibit the action of the enzyme. But, since the enzymes act upon the substrates and not upon other substances with which they combine, there seems to exist some closer relation between enzyme and substrate than between enzymes and other substances. Among the substances that combine with enzymes, and thus inhibit enzymation, are some which exercise a specific checking influence. In such cases there is probably some close relation between the enzyme and the inhibiting substance. As a matter of fact, the reaction between rennin and its specific anti-enzyme seems to take place in the same way as the colloidal reactions of non-specific nature and to be influenced by the same factors. But the kind of relation between the enzymes and their specific inhibitory substances has not yet been satisfactorily explained.

RECENT WORK ON THE PHYSIOLOGICAL PATHOLOGY OF GLYCOSURIA*

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THE advancement which medical knowledge has made during recent years can no doubt be attributed in large part to the closer affiliation which has developed between clinical and laboratory workers. Amongst the earliest attempts to apply the results of laboratory investigation in the clinic were those made on patients with diabetes, and although the progress achieved in the elucidation of the cause of this mysterious disease may not have been so striking as in the case of some other diseases, there has nevertheless been a steady accumulation of knowledge, which, when intelligently applied, is of immense value in its prophylaxis and treatment.

As attesting the value of laboratory investigation side by side with clinical, it is a noteworthy fact that the physicians who have been most successful in the treatment of diabetes are those who have kept themselves in constant touch with the work of both laboratory and clinic. Many of these men, such as Naunyn, Külz, Lepine, Pavy, Minkowski and von Noorden, have contributed materially to the advancement of our knowledge of the history of sugar in the animal body by laboratory experiment, while at the same time busily engaged in observing and treating cases of diabetes in clinical practice.

One result of the laboratory investigations has been to show that conditions exhibiting at least some of the symptoms of diabetes in man, more especially glycosuria, can be induced in animals by a multitude of causes, an experience which finds its clinical counterpart in the etiologic, in the therapeutic, and in the prognostic aspects of diabetes mellitus; etiologically, in

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the fact that such apparently distinct and separate influences as inheritance, nervous strain and over-indulgence in carbohydrate foods all predispose to the disease; therapeutically, in the undoubted benefit which certain cases derive from curtailment of the carbohydrate ingestion, whilst others benefit more from control of proteins; and prognostically, in the rapid development of the disease in some cases as compared with its gradual betterment in others. These facts would seem to indicate that in the clinic, as in the laboratory, we are dealing not with a distinct and separate pathological entity, but with a variety of conditions having only certain symptoms in common. It is indeed quite uncertain whether there is any variety of laboratory diabetes that can be strictly compared with any clinical form, for even in the case of the diabetes which is produced in animals by removal of the pancreas, there are so many dissimilarities from most of the severe clinical cases that a strict analogy cannot be drawn between the two. We shall see, however, that, in spite of these differences, many of the problems which the clinical observer has to face in treating the disease can be satisfactorily studied by work on animals, and many mistakes that might otherwise be made in applying new methods of treatment thereby avoided.

Facing such difficulties, how then are we to proceed in investigating the cause of diabetes? There is obviously but one way, and that is by collecting and carefully correlating all information we can regarding the causes which may produce the main symptoms of diabetes, either by careful clinical study in the case of man, or by well-reasoned and properly conducted experiments performed on lower animals.

Of the symptoms of diabetes, glycosuria has naturally been chosen as the most characteristic. It must be remembered, however, that since this symptom may depend merely on local interference with renal function, and since the essential derangements which are responsible for the development of the diabetic state may already be considerably advanced before any glycosuria makes itself evident, it is more reliable for most purposes to employ as our criterion of diabetes, not

glycosuria, but hyperglycæmia. But in doing this, we must be careful to bear in mind the physiological conditions which govern the relationship between hyperglycæmia and glycosuria. It has commonly been taught that the kidneys do not permit any, or only a trace, of the blood-sugar to escape into the urine until the percentage in the blood has risen to considerably above the normal level. The kidney has been compared in this connection to the overflow tube of a cistern, the contents of which, representing the blood-sugar, are ordinarily discharged by other paths. When for some reason these paths do not discharge quickly enough or when the inflow is too rapid, the level rises until the overflow is reached.

For many purposes this analogy has proved most useful, but it is far from being a strictly correct one. It explains why a temporary increase in blood-sugar, such as that produced by stimulation of the splanchnic nerves or by parenteral administration of sugar in laboratory animals, is unaccompanied by any demonstrable glycosuria until the blood-sugar level has risen to a certain height, after which the glycosuria runs more or less parallel with the hyperglycæmia. It also explains why there may be a considerable increase in the blood-sugar in healthy persons, as after a meal, but no glycosuria. It does not apply, however, in well-established diabetes in man, either in acute cases, in which there is usually much more sugar in the urine than would be expected from the amount present in the blood, or in chronic cases, in which there may be no demonstrable glycosuria, and yet a marked degree of hyperglycæmia.[1] Some change in the excretory function of the kidney must therefore be considered as one of the results of the diabetic state in man, a conclusion for which evidence is furnished by the observation that the same degree of hyperglycæmia causes a higher degree of glycosuria in a diabetic than in a healthy individual.[2]

But even for normal animals, the cistern model has been condemned as misleading because it fails to explain why feeding with increasing quantities of sugar, beyond the assimilation limit, does not cause all of the excess to be drained away in the urine. But this does not condemn its use, for it has recently been shown that it is not because the kidney

holds it back, but because the organism as a whole becomes stimulated to dispose of the sugar, that the excess fails to appear in the urine; thus, when varying amounts of sugar are fed to rabbits, the degree of hyperglycæmia which develops after each administration is but very little greater when those are excessive than when they are moderate.[3] Even when the kidney itself is healthy, the above parallelism obtains only provided there is no disturbance in its blood flow. Consequently, we find that the excretion of sugar into the urine is readily affected by changes in arterial blood-pressure. Thus, a certain degree of hyperglycæmia may cause marked glycosuria when the blood-pressure is normal, but none at all when it is subnormal.[1]

There are also certain forms of experimental glycosuria to which the above model does not apply, for they seem to owe their origin to no other lesion than one so affecting the kidney as to make it excrete large quantities of sugar from normal blood. These include not only the important form of experimental diabetes which is caused by phlorhizin but also that which occurs after poisoning by such metals as uranium, etc.[4] It has been thought possible that there may be a clinical variety of renal glycosuria. Such cases could be diagnosed with certainty only by comparisons between the sugar concentrations of blood and urine, although a strong suspicion of their existence would be furnished by finding that the ingestion of excess of carbohydrate food did not increase the glycosuria. It is very doubtful, however, if such purely renal cases exist.[5]

I need scarcely pause to point out that although phlorhizin glycosuria, in so far as it is unaccompanied by hyperglycæmia, is unlike the clinical varieties of glycosuria, this does not diminish the great importance of the studies which have been made by Lusk and his pupils concerning the nature of the faulty metabolism which it entails. Indeed I do not overshoot the mark when I assert that more accurate knowledge concerning the possible antecedents of dextrose in the animal body has been gleaned by investigations on this form of experimental glycosuria than by those made on any other, either experimental or clinical; for, by establishing a constant drain of dextrose from the blood, phlorhizin calls into play all the agencies which

are available in the organism for the manufacture of new supplies. It may be considered as the standard method for studies on gluconeogenesis.

In choosing hyperglycæmia as the most characteristic symptom of an abnormal state of carbohydrate metabolism, we must remember that it is not necessarily a sign of diabetes, for, besides the hyperglycæmia which develops in all diseases that are associated with asphyxial conditions, or with pyrexia,[6] a distinct increase in blood-sugar occurs as a physiological condition after the ingestion of any food that is rich in carbohydrates. Although this has been known for several years, it has only recently been made the subject of thorough investigation, both in the lower animals and in man, and with far-reaching results. Bang and his collaborators,[7] using a so-called micro-method, estimated the sugar concentration of the blood in rabbits during short intervals of time following the giving by stomach-tube of from 2–10 gm. of various sugars or starches. An increase in blood-sugar was evident in fifteen minutes, and it gradually reached a maximum which was about double that of the normal, in about an hour, then gradually fell to the old level, which was reached in about three hours. This post-prandial hyperglycæmia developed more quickly, and was more marked, in starved than in recently fed animals, and it became less and less evident when the administrations were repeated, until ultimately the 10 grammes of dextrose could be ingested without producing any effect on the blood-sugar. Galactose, maltose and saccharose, given in the same amounts, caused a similar rise, but the return to the normal level was delayed.

Corresponding results were obtained by Fisher and Wishart[8] after giving 50 grammes of dextrose by stomach-tube to dogs. A definite hyperglycæmia occurred during the succeeding hour, after which the blood-sugar quickly fell to normal, apparently in large part because of dilution of the blood by water absorbed from the tissues. At least it was found by making hæmoglobin estimations that during the hyperglycæmic stage the amount of water in the blood remained unchanged,

but that as the hyperglycæmia decreased, the blood became more diluted. As this occurred, there was evidence of increased combustion of carbohydrates, as shown by a rise of the respiratory quotient and by a greater energy output.

A most significant fact was brought to light, particularly by Bang's researches, namely, that 10 grammes of starch produced exactly the same effect on the blood-sugar as 10 grammes of dextrose, provided it was given to previously starved rabbits. Given to rabbits having food already in the stomach, the starch had little effect. Starch-rich foods, such as potatoes and turnips, produced the same results as starch itself; they readily caused hyperglycæmia on an empty stomach, but had little effect when the stomach was full. Evidently the rate with which the starchy food passes from the stomach to the intestines determines in large degree the extent to which it is likely to produce hyperglycæmia.

Before we seek for any application of these results on lower animals that may aid us in the diagnosis and treatment of diabetes in man, we must obtain evidence that in him similar changes in the blood-sugar accompany the ingestion of carbohydrates. Such caution is particularly necessary when we are dealing with carbohydrate metabolism, because the factors which govern the various stages in the breakdown of sugar may vary considerably in different animal groups. Even in so comparatively simple a condition as that which we are considering, the dog and the rabbit do not, for example, behave alike, for in the former animal not only is the hyperglycæmia which follows sugar ingestion less in degree than it is in the case of the rabbit, but there is a greater tendency for glycosuria to become established. Without actually trying, it would obviously, therefore, be very risky to assume that what occurs in the rabbit will also occur in man. Jacobson [2] has, however, recently furnished us with evidence that it does, for he found after giving 100 grammes of dextrose to normal persons before breakfast, that an increase was evident in the blood-sugar, even in so short a time as five minutes, and that the hyperglycæmia became more marked until in about thirty minutes

it had reached a maximum, after which it fell to reach its normal or even a subnormal level in about two hours.*

In eight out of fourteen of the persons employed in these investigations, examinations of the urine, voided at various short intervals after taking the sugar, revealed the presence of glycosuria, and the same was true in six out of fourteen, after giving starch, a fact which I consider to be one of the most important that has recently been contributed in the whole diabetes question; and this all the more so because practically the same results were obtained after giving an amount of white bread (167 grammes) capable of yielding 100 grammes of dextrose. The development of the hyperglycæmia became much slower when digestion was prolonged by giving a large quantity of butter along with the bread. No change was produced in the blood-sugar after feeding with proteins alone.

The conclusion that the only difference between sugar and starch, in so far as they influence carbohydrate metabolism, rests in their relative rates of absorption, is most strikingly confirmed by these observations and since, as is now well recognized, the object which we must aim at in the treatment of early cases of diabetes in man is the maintenance at as low a level as possible of the sugar content of the blood, it is evident from the above observations that the ingestion not only of sugar, but of starchy foods as well, requires careful regulation. Whatever carbohydrates are given must not only be well below the tolerance limit, but they should be such as are so slowly broken down to sugar that no unphysiological degree of hyperglycæmia is permitted to develop.

The decrease in glycosuria which opium causes in many diabetics may be due to the retarding influence of this drug on the emptying of the stomach. But apart from the use of opium, there are several ways in which we may possibly bring about the delay in the absorption of starch. Three of these are: (1) By mixing the starch with fats; (2) by choosing foods in which the starch grains are naturally more or less protected from digestion; (3) by choosing varieties of starches that are themselves

* This succeeding hypoglycæmia, although interpreted in a different way, had previously been recognized by Frank.

only slowly digested. Regarding the last point it should be mentioned that there exists remarkably little information regarding the digestibility of different starches by diastase. Information on this point is, however, gradually accumulating.

These considerations naturally lead us to the question as to whether the undoubted benefit which certain cases of diabetes derive by feeding with oatmeal, in the absence of other carbohydrates and of animal proteins, may not depend on the slow rate at which the starch in this food is digested and absorbed. Cohnheim and Klee have shown by observations on dogs having duodenal fistulas that oatmeal causes very much less pancreatic juice to be secreted than when an equivalent amount of wheaten bread or potatoes is given.[9] Although these two facts concerning oatmeal, namely, that it calls forth but little pancreatic secretion, and that it is a readily tolerated form of starchy food in diabetics, seem to be most simply correlated in the above manner, yet we must not overlook another possible explanation for the benefit of the oatmeal cure, namely, that by holding down the intestinal secretion of pancreatic juice, a better chance is given the pancreas to furnish the internal secretion or hormone whose deficiency is thought to be in some way associated with the cause of the disease. The well-known fact that the oat cure is not likely to meet with any success unless such foodstuffs as animal proteins and wheat starch are simultaneously withheld, is readily explained on account of the readiness with which these foods excite pancreatic secretion. In the same manner, the benefit of alkali treatment may be explained.

It is more probably in some such manner as that discussed above that oatmeal acts in diabetes than, as has been suggested, by its furnishing to the organism some vitamine necessary for the utilization of sugar. I refer to some such substance as has been shown to be necessary for the prevention of beriberi in rice feeders, and to be important for proper increase in body weight in growing animals.

In so far as these laboratory observations apply to the management of early cases of diabetes, we may sum up by saying that the earliest indication of derangement in carbohydrate

metabolism is that the post-prandial hyperglycæmia is greater, and persists longer than it should, becoming progressively more and more marked, until ultimately the separate periods fuse into one continuous hyperglycæmia, which for some time is probably maintained at a fairly constant level by overflow of the excess of sugar into the urine. But ultimately the kidneys, suffering possibly because of the constant toxic influence of the excess of sugar, become less permeable towards it, and again the hyperglycæmia begins to mount. Although the literature does not, so far as I am aware, contain a sufficiently complete series of records of blood-sugar estimations in diabetes, especially in its earlier stages, to justify the positive assertion that the above course is run by every case, yet very strong evidence that it is so is furnished by the observation that glycosuria is evident in early stages of diabetes only after taking food. The mixed twenty-four-hour specimen, when tested, however carefully, by the various qualitative tests for sugar, may be normal, whereas the specimen voided during the hour or two immediately following a meal may give a distinctly positive test. *For practical purposes post-prandial glycosuria must undoubtedly be considered as the initial symptom of diabetes,* provided, of course, that excessive amounts of sugar have not been taken. Its occurrence should serve as an indication that the diet must be altered, for although we cannot cure a late case of diabetes, we can, by preventing glycosuria, if not indefinitely, yet for a long time, retard the development of incipient cases into those of a more serious character.

In employing hyperglycæmia as our most trustworthy criterion of disturbed carbohydrate metabolism in experiments on anæsthetized animals, many precautions must be taken so as to avoid, or at least discount, the effects which fright, anæsthesia, body temperature, etc., may have in causing that condition.[10] [11]

The above facts concerning the behavior of the blood-sugar following carbohydrate ingestion bring us to the consideration of one of the most puzzling questions regarding the history of carbohydrates in the animal body: What becomes of the sugar immediately after its ingestion? Recent work compels us to

modify our older ideas that the greater proportion of it becomes immediately deposited as glycogen, for the sugar may disappear from the blood without there being any very evident increase in the amount of this substance in the liver and muscles. Thus by quickly injecting 0.5–1.0 gm. of dextrose per kilo of body weight in 10 per cent. solution into the ear vein of rabbits, Bang[12] found that although there was an immediately succeeding increase in the amount of sugar in the blood, this did not last for long and could not account for more than 10 per cent. of the injected sugar. The question is: What becomes of the 90 per cent. of sugar which disappears? It did not escape into the urine, although a small degree of glycosuria might develop, especially when the injections were rapidly made. Nor could more than about one-fifth of the amount injected be recovered as glycogen and sugar from the liver.

Similar examination of the various other organs, including the muscles, the intestines and the connective tissues, accounted for some of the disappearing sugar, but after allowing for the almost insurmountable difficulties involved in such estimations, Bang concludes that only about one-half of the injected sugar had become assimilated in such a way as to be recoverable, either as free sugar in the tissues and tissue fluids or as glycogen in the liver.

The power of the organism to dispose of an excess of dextrose in the absence of the liver has been further demonstrated by experiments in which it was found that dextrose when injected into the blood of eviscerated animals,[13] or into animals in whom the aorta had been ligated above the cœliac axis,[14] disappeared almost as rapidly, in relation to the mass of tissue left in the animal, as in intact animals. It is apparently into the muscular tissues that a great part of this sugar disappears, for in dead animals, as Meltzer and Kleiner have shown, a similar disappearance of intravenously injected sugar can be accounted for by the increase in the sugar content of the muscles.

In what manner does the sugar that we cannot recover either as free sugar or as glycogen become disposed of? There are in general two possibilities: Either it may become built up

into substances other than glycogen, or it may become rapidly broken down. Concerning the first of these, the sugar may become attached to other substances and thereby lose its reducing properties, or several molecules may fuse together so as to form condensation products, which are possibly not included in the glycogen precipitates, and which nevertheless fail to exhibit the reactions of dextrose. The evidence which justifies us in thinking of these possibilities has been furnished by Lepine[15] and Pavy,[16] and more recently by Levene and Meyer.[17] The former workers, as an outcome of their extended researches, have concluded that a considerable proportion of the sugar of the blood exists in a more or less firmly combined condition, from which it can be dislodged only by energetic hydrolysis, as by heating with strong mineral acids. Levene and Meyer have shown that, under certain conditions, tissues or their juices, when incubated along with dextrose, may cause non-reducing condensation products to be formed.

Regarding the second possibility there is no doubt that part of the excess of sugar becomes completely oxidized within a comparatively short time after its entry to the blood[18]; a portion may, however, become incompletely broken down, thus leaving substances which are subsequently only slowly oxidized, or which may possibly be excreted or disposed of in other ways. In this connection the recent brilliant series of researches of Embden and his pupils[19] showing that lactic acid accumulates at the same time that sugar or glycogen disappears from an artificially perfused liver, or even from blood kept at body temperature outside the body, offers us results which are at least suggestive.

These observations regarding the mysterious disappearance of injected dextrose in the organism call to mind another very similar mystery. This concerns the disappearance of glycogen from the liver without any corresponding increase in the sugar percentage in the blood, which occurs more particularly in poisoning by hydrazine[20] or phosphorus,[21] but also to a certain extent after giving adrenalin[22] to some animals, and even during ether anæsthesia in dogs after feeding with large quantities of sugar.[1] What becomes of the enormous quantities of glycogen that disappear under these conditions is at present entirely unknown.

One other question concerning the sugar in the blood remains to be discussed, and that is its condition, whether free or combined. This does not mean combined in the sense that it is no longer included in our blood-sugar estimations, but combined in such a manner as to be readily split off. I do not intend to weary you with all the outs and ins of this most troublesome question, but I must at least pause to explain its significance, and to lay before you some of the most recent work which has been brought to bear on its elucidation. The hypothesis is that most of the dextrose exists in some sort of loose combination, possibly of physical chemical nature, with certain of the blood constituents, and that a small fraction only is free. It is further supposed that it is only after it has become thus combined that the sugar can be utilized by the tissue cells. Of the older writers, it was Pavy who most insistently upheld this view, and there are few who have not at one time or another expressed some adherence to it. The most recent advocate is Allen,[24] whose most exhaustive treatise on diabetes is indeed built around the idea that all the circulating sugar of the normal body exists in some sort of loose combination, in which form it is assimilated by the tissues.

It is impossible to settle this question by chemical investigation of the blood: even the brilliantly conceived experiments of Michaelis and Rona fail to do so. In their experiments it was found that when samples of sterile unclotted blood were dialyzed against a series of isotonic solutions each containing a different percentage of dextrose, diffusion of dextrose in one or other direction occurred in all save one case, namely, in that in which the percentage of dextrose in the outside fluid was exactly equal to the total sugar content of the blood. Such a result can be interpreted only by assuming that all of the sugar exists in the blood in a freely diffusible state, a conclusion which is supported by the results of the colloidal precipitation experiments of the same workers.[1] The chemical evidence so far as it goes thus favors the view that the blood-sugar exists in a free state. On what grounds, then, do so many authorities insist that the sugar is practically all combined? They do so

on biological grounds, namely, because of the difficulty of explaining in any other way why the blood-sugar does not constantly leak into the urine. Pavy, for example, insists that if it were free in the blood, sugar, like any other body of small molecular weight, would flow off in the urine; and exactly the same line of argument is contained in Allen's statement that "in normal animals all sugar not injected intravenously reaches the circulation in combination with a colloid," so that "it behaves as a typical colloid, not only in failing to pass into the urine, but in depressing the excretion of this fluid. When, however, sugar is added directly to the blood, it fails to become thus combined, with the result that like any other crystalloid it escapes in the urine and stimulates diuresis." These conclusions are based on the observation that when dextrose is given intravenously to a normal animal, it causes diuresis, whereas anuria results when it is given in any other way. To explain these differences it is supposed that the sugar molecule in passing through the endothelial wall of the capillaries combines with some substance, as a result of which the sugar becomes available for incorporation in, and utilization by, the tissues, which in the free state it is not. This substance is supposed to be related to the internal secretion of the pancreas, and it has been called an amboceptor to indicate that it links the dextrose molecule to the tissues.

But the upholders of these views do not stop short in merely explaining how sugar circulates in the normal animal. They go further, for they tell us that "deficiency of the amboceptor is diabetes"; in other words, they assert that the essential lesion in diabetes is a failure of the sugar that is absorbed from the intestine to become united with amboceptor, as a consequence of which it circulates in a free state and is not available for combustion in the tissues. This free sugar, acting like a crystalloid, stimulates the kidney to diuresis and itself escapes by this path. Allen has very cleverly defended this hypothesis against most of the criticisms that could be brought against it, although I for one am not yet quite convinced that the striking differences in diuretic effect which he found according to the manner of

administration of the sugar may not be largely a question of the rate at which the sugar becomes added to the blood. It is at least significant that when the intravenous injections of sugar are made very slowly, the renal function behaves exactly as it does when the slower subcutaneous or intestinal paths of absorption are traversed.[25] The remarkable disappearance of dextrose from the blood which we have seen occurs when it is intravenously injected in eviscerated animals shows also that incorporation with the tissues is possible without any amboceptor attachment in Allen's sense.*

Although of the very highest importance as showing the manner in which carbohydrates behave immediately after they are ingested, the researches which we have so far considered bring no light to bear on the nature of the disturbances which may cause excessive amounts of dextrose to pile up in the blood, independently of increased ingestion of carbohydrates. Their object is to inform us as to how excess of ingested sugar may be disposed of, but not to explain why in severe forms of diabetes there comes to be a constant and uncontrollable new production of sugar, or gluconeogenesis, in the organism itself. To do this our attention must be turned to a study of the conditions which bring about excessive sugar production in laboratory animals. At the very outset of such an investigation, however, we are met with a serious difficulty on account of the multiplicity of experimental conditions which may cause hyper-

* It has been common to consider that glycogen is nothing more than a storage material for the carbohydrates which the organism does not require for its immediate uses. Some have thought, however, that glycogen, besides being a storage material, is likewise a necessary preliminary stage in the metabolism of sugar; in other words, that no sugar is burnt unless it has passed through a glycogen stage. Time will not permit me to say more with regard to this question than that there is so far no conclusive evidence which would enable us to decide it one way or the other. That liverless animals can burn injected sugar, or that isolated muscular tissues use it, is no proof that the sugar has been directly used, for the muscles contain glycogen. On the other hand, the glycolytic powers of blood are exactly the same towards ordinary dextrose as towards dextrose produced by acting on glycogen by the diastase of the blood or liver.[26]

glycæmia and glycosuria. It becomes necessary to adopt some classification, the most suitable one for our purpose being into transient and permanent forms of hyperglycæmia. There is practically but one form of permanent experimental hyperglycæmia, namely, that which follows complete removal of the pancreas, and since the behavior of the animal in this condition is in many respects like that of the severely diabetic patient, it has been chosen as the experimental prototype of the clinical disease. Most forms of transient experimental hyperglycæmia are more or less dependent upon stimulation of those portions of the nervous system that exercise a control over the glycogenic function of the liver. The cause of the difference between the two forms is, therefore, that in the transient, the excess of sugar is derived from no other source than the glycogen reserves of the liver and muscles, whereas in the permanent, it is derived from this source only until no more glycogen remains, after which it comes from protein or possibly from some varieties of fat. An excessive hepatic glycogenolysis occurs as the first stage in both types of experimental diabetes, so that an understanding of the nature of the factors which control the glycogenic function of the liver and of the conditions which may lead to a failure of this control is fundamental to intelligent investigation of the diabetes problem.

Although it has so far been impossible by any kind of disturbance of the nervous system to create a diabetic state in laboratory animals which is comparable with that following removal of the pancreas, yet there is abundance of clinical evidence to show that a certain nervous element does enter into the causation of diabetes mellitus. The frequent occurrence of diabetes in those predisposed by inheritance to neurotic conditions, or in those whose daily habits entail much nerve strain, and the aggravation of the symptoms which is likely to follow when a diabetic patient experiences some nervous shock, all point in this direction. Diabetes is very common in locomotive engineers and in the captains of ocean liners, that is to say, in men who, in the performance of their daily

duties, are frequently put under a severe nerve strain. It is apparently increasing in men engaged in occupations that demand mental concentration and strain, such as in professional and business work. Cannon and his collaborators[27] found glycosuria in four out of nine students after a severe examination, but in one of them only after an easier examination. In the urine of twenty-five members of a famous football squad, sugar was found present in twelve immediately after a keenly contested game. Anxiety and excitement were its cause, for five of the twelve were substitutes who did not get into the game.

Although these nervous conditions, by excitement of hepatic glycogenolysis, produce at first nothing more than an excessive discharge of sugar into the blood, a condition which is exactly duplicated in our laboratory experiments by stimulation of the nerve supply of the liver, their repetition gradually leads to the development of a permanent form of hyperglycæmia. To prevent the repetition of these transient hyperglycæmias must be our aim in the treatment of the early stages of the disease. This may be done partly by control of the diet and partly by removal of any possible cause of nerve strain.

The simplest experimental condition which illustrates the relationship between the nervous system and the blood-sugar is electrical stimulation of the great splanchnic nerve in animals in whom, by previous feeding with carbohydrates, a large amount of glycogen has been deposited in the liver. By examining the blood as it is discharged into the vena cava from the hepatic veins, the increase in blood-sugar is very evident in from five to ten minutes after the first application of the stimulus, but it is not until later that a general hyperglycæmia becomes evident. The conclusion which we may draw from these results is that the splanchnic nerve contains efferent fibres controlling the rate at which glycogen becomes converted to dextrose. The centre from which these fibres originate is situated somewhere in the medulla oblongata, for the irritation that is set up by puncturing this portion of the

nervous system yields results similar to those which follow splanchnic stimulation. It is natural to assume that there must be afferent impulses running to this centre. Such have indeed been described in the vagus nerves, but their demonstration is by no means an easy matter on account of the disturbance in the respiratory movements which stimulation of the central ends of these nerves entails. The changes which such disturbances bring about in the aëration of the blood may in themselves be responsible for the hyperglycæmia. This much can at least be said, that when the respiratory disturbances are guarded against, as by intratracheal insufflation of oxygen, vagal hyperglycæmia is much less marked, if not entirely absent. But this question requires more thorough investigation.

Returning to the efferent fibres in the sphanchnic nerves, two explanations are possible for the increased glycogenolysis which results from their stimulation: either, they exercise direct control over the glycogenic functions of the hepatic cells, or they govern the discharge from some ductless gland of an internal secretion of hormone which excites the glycogenolytic process. From their anatomical position the adrenals are to be thought of in this connection, and evidence that they really do perform such a function has seemed to be furnished by the fact that after extirpating them, splanchnic stimulation no longer produces hyperglycæmia,[28] nor indeed does puncture of the medulla.[29] Not only this, but there is no doubt that the nervous system, acting by way of the splanchnic nerves, does exercise a control over the discharge of the internal secretion of the adrenal gland,[30] and moreover that extracts of the gland, which we must suppose contain the internal secretion, cause hyperglycæmia.

But on theoretical grounds alone, certain difficulties immediately present themselves in accepting this as the mechanism by which the nervous system controls the sugar output of the liver, for if increased sugar formation in the liver is entirely dependent upon a discharge of adrenalin into the blood, why should the adrenalin have to traverse the entire circulation

before it reaches the liver? There are, besides, certain experimental facts which do not conform with such a view. After complete severance of the hepatic plexus, stimulation of the splanchnic nerve does not cause the usual degree of hyperglycæmia; whereas electrical stimulation of the peripheral end of the cut plexus does cause it. On the one hand, therefore, there is evidence that stimulation of the efferent nerve path above the level of the adrenal glands has no effect on the sugar production of the liver in the absence of these glands, and on the other, we see that when they are present, stimulation of the nerve supply of the liver is effective, even though the point of stimulation is beyond them. There is but one conclusion that we may draw, namely, that the functional integrity of the efferent nerve-fibres that control the glycogenolytic process of the liver depends on the presence of the adrenal glands, very probably on the hormone which these glands secrete into the blood. This conclusion is corroborated by the fact that stimulation of the hepatic plexus, even with a strong electric current, some time after complete removal of both adrenals, is not followed by the usual degree of excitement of the glycogenolytic process.

I have brought these experiments before your notice because of the light which they throw on the relationship between the nervous and at least one form of hormone control of the sugar output of the liver. They indicate that when a sudden increase of blood-sugar is required, the glycogenic centre sends out impulses which act not only on the hepatic cells, but which simultaneously influence the adrenal glands in such a manner as to facilitate the passage of nerve impulse on to the liver-cells. The fact elicited by Cannon and his co-workers and by Elliott that hypersecretion of adrenalin occurs during conditions of deep emotion, fright, fear, etc., becomes doubly interesting when we consider that these conditions also lead to hyperglycæmia. It seems natural enough to assume that the discharge of nervous energy which occurs in man in emotional states likewise affects both the adrenal activity and the glycogenic function of the liver.

In this connection, the recent experiments by Miculicich [31] are interesting, for he found that after paralysis of the sympathetic nerve terminations by means of ergotoxin, no glycosuria, and only a slight degree of hyperglycæmia, was caused either by adrenalin, whose locus of action is the peripheral nerve terminations, or by diuretin, which acts on the glycogenic centre. After injecting hirudin, adrenalin failed to cause hyperglycæmia, but diuretin still caused it, thus indicating that the hyperglycæmia produced by stimulation of the glycogenic centre does not occur because of a hypersecretion of adrenalin. As this author himself points out, however, great caution must be taken in drawing conclusions from such experiments.

We are as yet quite in the dark as to the mechanism by which the nerve impulse brings about increased glycogenolysis. Many possibilities must be borne in mind. It will be remembered that the glycogen is deposited in the cytoplasm of the liver-cell in somewhat irregular masses, being possibly combined with some form of sustentacular substances. Our present problem is to explain how it comes about that these masses of glycogen are suddenly discharged from the cells as dextrose when the nerve supply is stimulated. The first thing to be thought of is an increase within the liver-cell of the ferment which hydrolyzes glycogen to dextrose. But this does not seem to be the mechanism, for it is impossible to show that there is any more of this ferment in an extract prepared from a liver in which active glycogenolysis is in progress than in one from a liver that is quiescent in this regard.[32]

It is well known that enzyme activities may become most markedly altered by slight changes in the chemical and physical nature of the environment in which they act. Diastatic enzymes are particularly susceptible to the reaction of their environment, a very slight degree of acidity, as measured by the H-ion concentration, favoring, whereas a trace of alkalinity depresses their activity. That this favoring action of acid may occur in the case of the diastase of the intact liver has been well established, partly by studying the effect which is produced on sugar tolerance by administering acids through the alimentary canal,[33] and partly by studies on post-mortem glycogenolysis.[34] It might be thought, then, that the nerve

impulse sets free in the liver-cell a certain amount of acid, which, being produced locally, would encourage diastatic action before it became neutralized. This liberation of free acid could obviously be dependent upon a curtailment in the blood supply of the hepatic cell, thus producing a local accumulation either of carbonic or of other less completely oxidized acids. That vascular changes do occur in the liver when the hepatic nerves are stimulated,[35] and that such changes may in themselves alter the rate of glycogenolysis, are well established facts.[36] The accelerating effect of adrenalin on the glycogenolysis which is occurring in a perfused liver outside the body[37] may be explained as due to limitation of blood supply on account of vasoconstriction. But until such a local production of acid is actually demonstrated in the liver-cell, it would be unsafe to give this hypothesis too much weight.

Another possibility is that the glycogen, on account of combination with a sustentacular material, is ordinarily removed from the influence of the intrahepatic glycogenase, and that the nerve impulse acts by disrupting this combination and thus exposing the glycogen to attack. In contrast to the preceding, we may call this the mechanical hypothesis. It deserves serious consideration, for it has been shown that very little postmortem glycogenolysis occurs in the intact liver of frogs in winter—even though at this time the organ contains an excess of glycogen—but becomes marked when the liver is broken down by mechanical means.[38]

There is indeed some evidence that the glycogen may be expelled as such from the liver-cell into the neighboring vessels, where it is converted to sugar by the glycogenase, which the fluid of the vessels contains. Thus, Ishimori,[39] working in Hofmeister's laboratory, found by microscopical examination of sections of the rabbit's liver stained for glycogen by Best's carmine method that some time after piqûre had been performed, the glycogen masses were found to have been irregularly discharged from the lobules, some of them being actually present as such, not only in the interlobular lymph-spaces, but also in the venous radicals, whereas that which remained in

the lobule was distributed in an irregular fashion. The conclusion seems justified that the nerve impulse causes glycogen itself to be discharged from the cell into the hepatic vessels, where, being freely exposed to the glycogenolytic enzymes therein present, it very quickly becomes hydrolyzed to sugar. The extrusions of zymogen granules and vacuoles are quoted as examples of processes of a similar nature occurring in other cells. The presence of colloid in the vessels of the thyroid gland is another example.

There are some other observations which could be explained as due, in part at least, to such a process. For example, Lepine has frequently insisted that the blood, immediately after leaving the liver, contains less actual sugar but more of some substance, which he does not specify, but calls "virtual" sugar, than the blood of the carotid artery. He believes that this "virtual" sugar becomes converted to actual sugar during the time that the blood takes to circulate through the lungs. The "virtual" sugar may be some intermediary substance between glycogen and dextrose, some dextrin, perhaps, which is precipitated along with the proteins in preparing the blood for estimation of sugar. Then again, the remarkable disappearance of glycogen from the liver, without any accompanying increase in blood-sugar, which we have already noted occurs in poisoning by phosphorus or hydrazine, or even in certain animals after adrenalin or ether, may possibly be associated in some way with an extrusion of glycogen which the blood fails to convert to sugar. I have sought for the presence of this "virtual" sugar in the blood issuing from the liver after adrenalin poisoning and after splanchnic stimulation, but so far have failed to demonstrate its presence.[26]

To sum up, we may conclude that the nerve impulse that brings about an increased discharge of dextrose from the liver does so either by producing in the liver-cell some chemical change which facilitates the action of the glycogenase on the glycogen, or by causing the glycogen masses to become dissociated from the protoplasm so that they become susceptible to the intracellular enzymes, or become extruded from the cell so that they are attacked by the glycogenase which exists in the neighboring lymph- and blood-vessels.

The forms of hyperglycæmia which are dependent upon excessive hepatic glycogenolysis, and which do not last after the supply of glycogen is exhausted, are very numerous. They include the hyperglycæmia pro-

duced by drugs and by asphyxial conditions. The exact part of the controlling mechanism which is acted on may vary considerably. We have seen, for example, that diuretin is believed to act specifically on the glycogenic centre, whereas adrenalin acts on the nerve terminations. In mild degrees of asphyxia, it is probable that irritation of the glycogenic centre by asphyxial products in the blood accounts for the hyperglycæmia, whereas when the asphyxia is sufficiently severe to cause a distinct increase in the hydrogen ion concentration of the blood, direct action on the glycogenolytic process in the liver occurs. Although many believe that the glycosuria which develops after injection of sodium salts is dependent either upon a change in the permeability of the kidney towards sugar or upon asphyxial disturbances, it is possible that the sodium may have a certain influence on the glycogenolytic process itself. In attempting to explain why any drug or experimental condition produces hyperglycæmia, close watch must always be exercised over the temperature of the blood, since changes in this markedly affect the glycogenolytic process in the liver.[36]

But there is only one form of experimental diabetes, namely, that due to removal of the pancreas, that can be considered as analogous with the severer forms of the disease in man. It is true that disturbances in carbohydrate metabolism, sometimes severe enough to cause glycosuria, are also produced by disturbances in the functions of other ductless glands, for example, by removal of more than two parathyroids, or by conditions which cause a hypersecretion of the posterior lobe of the pituitary gland; but it has not been shown that permanent diabetes, as met with in clinical practice, bears any relationship to lesions in these glands. On the other hand, experimental pancreatic diabetes can be made to simulate very closely the disease in man. This was first demonstrated by Sandemeyer, who found that by removing not all but the greater part of the pancreas, the animals for some months, if at all, were only occasionally glycosuric, but later became more and more frequently so, until at last the condition typical of complete pancreatectomy supervened. Similar results have more recently been obtained by Thiroloix and Jacob, in France, and by Allen[40] in this country. These investigators point out that different results are to be expected according to whether the portion of pancreas which is left does, or does not, remain in

connection with the duodenal duct. When this duct is ligated, atrophy of any remnant of pancreas that is left is bound to occur, and this is associated with rapid emaciation of the animal, diabetes and death. When the remnant surrounds a still patent duct, a condition much more closely simulating diabetes in man is likely to become developed, one, namely, in which there is, for some months following the operation, a more or less mild diabetes, which, however, usually passes later into the fatal type. It is, of course, difficult to state accurately what proportion of the pancreas must be left in order that this interesting condition may supervene. Leaving a remnant amounting to from one-fifth to one-eighth of the entire gland is commonly followed by a mild diabetes, whereas if only one-ninth or less is left, a rapidly fatal type develops. As in clinical experience, the distinguishing feature between the mild and the severe types of experimental pancreatic diabetes is the tolerance towards carbohydrates. In the mild form, no glycosuria develops unless carbohydrate food is taken; in the severe form, it is present when the diet is composed entirely of flesh. It is thus shown that " by removal of a suitable proportion of the pancreas, it is possible to bring an animal to the verge of diabetes, yet to know that the animal will never of itself become diabetic. . . . Such animals therefore constitute valuable test objects for judging the effects of various agencies with respect to diabetes " (Allen). It therefore becomes theoretically possible to investigate other conditions which will have an influence similar to removal of more of the gland, or, to study conditions which might prevent the incidence of diabetes, even though this extra portion of pancreas is removed. From the work which he has already done Allen believes that he has sufficient evidence to show that the continued feeding with excess of carbohydrate food will surely convert a mild into a severe case, and in one experiment he succeeded in bringing about the same transition by performing puncture of the medulla, that is, by creating an irritative nervous lesion. By none of the other means usually employed to produce experimental glycosuria could

the bordering case be made diabetic, although one such animal became acutely diabetic after ligature of the portal vein. To the clinical worker the value of these results lies in the fact that they furnish experimental proof that a so-called latent case of diabetes, that is, one that has a low tolerance value for carbohydrates, may be prevented from developing into a severe case by proper control of the diet. Attempts to show whether there are any conditions which might bring about improvements in animals that were just diabetic have not as yet been sufficiently made to warrant any conclusions that could help us in the treatment of human cases. The encouragement of the internal pancreatic secretion by diminution of that discharged into the intestine has already been alluded to.

The certainty with which diabetes results from pancreatectomy in dogs, and the frequent occurrence of demonstrable lesions in the pancreas in diabetes in man, leave no doubt that this gland must be in some way essential in the physiological breakdown of carbohydrates in the normal animal. But how, we cannot at present tell. All we know is that the first change to occur after the gland is removed is a sweeping out of all but a trace of the glycogen of the liver, although the muscles may retain theirs; indeed, in the cardiac muscle there may be more than the usual amount.[41] Nor can any glycogen be stored in the liver when excess of carbohydrates is fed. After the glycogen has disappeared, a new process sets in, namely, that of gluconeogenesis, which, as its name indicates, consists in a manufacture of new sugar out of other than carbohydrate molecules. It is possible that more or less gluconeogenesis occurs in the animal body in health, and that the process merely becomes exaggerated in diabetes. Be that as it may, the fact remains that in this disease the tissues come to melt away into sugar, and all the symptoms of acute starvation, associated with certain others that are possibly due to a toxic action of the excess of sugar or of other abnormal products in the blood, make their appearance.

So far it might be permissible to consider an overproduction of dextrose as the cause of the hyperglycæmia of pan-

creatic diabetes, just as we have seen it to be of these forms of hyperglycæmia that are due to stimulation of the nervous system. But this cannot be the case, for another very definite abnormality in metabolism becomes evident, namely, an inability of the tissues to burn sugar. This fact is ascertained by observing the respiratory quotient, which, you will remember, is the ratio between the volume of CO_2 expired and the oxygen absorbed. When dextrose is added to the blood in a normal animal, the quotient rises almost if not entirely to unity, but however much may be added in the case of a completely diabetic animal, no change occurs.[47] Some investigators have endeavored to explain this lowering of the quotient in other terms than that it indicates an inability to burn sugar, but the recent work of Verzár and of Murlin[42] removes all doubt about the matter.

There are, therefore, two essential disturbances of carbohydrate metabolism in diabetes, (1) overproduction of sugar and (2) abolition of the ability of the tissues to use it. It becomes important for us to see whether the tissues exhibit this inability to use sugar when they are isolated from the animal, for if they should, a much more searching investigation of the essential cause of their inability would be possible than is the case when they are functioning along with the other organs and tissues. I need not remind you of the earlier experiments of Lepine and his pupils, who thought that diabetic did not possess the glycolytic power of normal blood; or of those of Cohnheim, who believed that mixtures of the expressed juices of muscle (liver) and pancreas, although ordinarily destroying dextrose, failed to do so when they were taken from a diabetic animal. The failure to show a depression of glycolytic power by these methods prompted Knowlton and Starling[43] to investigate the question whether any difference is evident in the rate with which dextrose disappears from a mixture of blood and saline solution used to perfuse a heart outside the body, according to whether the heart was from a normal or a diabetic dog. In the first series of observations which these workers made, it was thought that the heart of a

normal animal used dextrose at the rate of about 4 mg. per 100 grammes of heart substance per minute; whereas that of a diabetic (depancreated) animal used less than 1 mg. If such striking differences in the rate of sugar consumption could make themselves manifest for so relatively small a mass of muscular tissue as that of the heart, it is permissible to assume that a much more striking difference could be demonstrated when the perfusion fluid is made to traverse all or practically all of the skeletal muscles, as well as the heart. For this purpose an eviscerated animal may be employed, that is, one in which the abdominal viscera are removed after ligation of the cœliac axis and mesenteric arteries, and the liver is eliminated by mass ligation of its lobes. To avoid the necessity of anæsthesia the cerebral vessels are also tied off. R. G. Pearce and I[44] have found that the rate at which dextrose disappears from the blood in such a preparation, although very irregular, is in no way different in completely diabetic as compared with normal dogs. We were thus unable to confirm any of Knowlton and Starling's earlier conclusions. Patterson and Starling[45] have subsequently shown that a serious error was involved in the earlier perfusion experiments, partly on account of a remarkable but irregular disappearance of dextrose from the lungs, and partly because the diabetic heart may contain a considerable excess of glycogen, from which its demands for sugar may be met without calling upon that of the perfusion fluid. These observations have made it evident that conclusions regarding the rate of sugar consumption in the tissues cannot be drawn from the rate of the disappearance of this substance from the blood, unless determinations are simultaneously made of the amounts of glycogen in the tissues before and after the perfusion.[46] The property of the muscles to take up excess of sugar from the blood, which Meltzer and Kleiner have recently investigated, further illustrates the difficulties of work of the above nature.

The fact that on the one hand the normal animal, as judged from the respiratory exchange, burns more sugar when an excess is present in the blood, while the diabetic animal fails

to do so,[47] but excretes the excess instead, does not carry us very far in deciding whether the failure to burn the sugar is dependent upon a fault resident in the consuming tissues themselves, or upon one affecting some organ whose function it is to prepare the sugar molecule for utilization. All we really know at present is that the depancreated animal cannot burn sugar, but, encouraged by the fruitful outcome of researches on the ductless glands, we have come to assume that this inability is because the removal entails the loss to the organism of some internal secretion or hormone which is necessary for sugar combustion. This is, however, by no means the only way by which the results can be explained, for we can assume that the pancreas owes its influence over sugar metabolism to some local change occurring in the composition of the blood as this circulates through the gland; a change which is dependent upon the integrity of the gland and not upon any one enzyme or hormone which it produces. This view must be kept in mind. It is obvious that the results of removal of the gland could be explained in terms of either view, and indeed there is but one experiment which would permit us to decide which of them is correct. This consists in seeing whether the symptoms which follow pancreatectomy are removed, and a normal condition reëstablished, when means are taken to supply the supposed missing internal secretion to the organism; if they should be, conclusive evidence would be furnished that it is by "internal secretion" and not by "local influence" that the gland functionates.

The experiments have been of two types; in the one, variously prepared extracts of the gland have been employed, and in the other, blood which is presumably rich in the internal secretion. The most recent work with pancreatic extracts has been performed by Knowlton and Starling, by Verzár, and by Murlin and Kramer. Although the first mentioned workers have thought that their earlier experiments pointed to an improvement in the sugar-consuming powers of the isolated diabetic heart when pancreatic extracts were mixed with the perfusion fluid, more recent investigation, as we have seen, has

shown that the complexity of the experimental conditions renders such a conclusion untenable. It has been unreservedly withdrawn by Patterson and Starling. Neither Verzár nor Murlin and Kramer were able to find that injection of pancreatic extracts into a depancreated animal produced any effect on the respiratory quotient, although Murlin and Kramer did observe that injections of extracts of pancreas and duodenum produce a temporary fall in the dextrose excretion in the urine. More thorough investigation of the cause of this apparent anti-diabetic effect, however, revealed the fact that it was due to the alkalinity of the extract. Alkali temporarily decreases and acids increase the sugar excretion in the urine in diabetic animals, possibly by the influence which they have in accelerating or depressing glycogenolytic activity (see p. 192). Apart from the conclusions which they permit us to draw, these experiments are of great importance in cautioning us as to the extreme technical difficulties which present themselves in attacking this problem.

Nor have the experiments with blood transfusions yielded results that are any more satisfactory. In undertaking these experiments it is of course assumed that the internal secretion is present in the blood, and that if this blood be supplied to an animal suffering from diabetes because of the loss of its pancreas, it will restore it to a non-diabetic state. The most important experiments have been performed by Hedon, Forschbach, Carlson and Drennan,[1] Murlin and Kramer,[34] Woodyat[48] and Verzár and Fejér.[47] It were useless for me to review the results of each observer. The general conclusion which may be drawn from their researches as a whole is that there is no evidence that the blood of a normal animal, even when it is from the pancreatic vein, contains an internal secretion that can restore to a diabetic animal any of its lost power to utilize carbohydrates. When the extent of glycosuria alone is used as the criterion of the state of carbohydrate metabolism, serious errors in judgment are likely to be drawn. The condition of the blood-sugar and the extent and character of the respiratory exchange are the most reliable indices.

In this lecture I have endeavored to do no more than pick out a few of the researches that have interested me most during the past two years, and I have been compelled, perhaps somewhat arbitrarily, to exclude from my review, amongst other things, the extremely interesting and important work which has in this time been done on the nature of the chemical processes that are responsible for the new formation of sugar (gluconeogenesis) in the diabetic animal, and for the formation of acetone bodies which so regularly make their appearance in the later stages of the disease.

We may hope that the outcome of the studies which Ringer has recently prosecuted in this direction will be the successful control by therapeutic means of the development of the acetone bodies which occurs in the later stages of diabetes, just as the researches which I have attempted to review this evening indicate in what way we may hope to control the disease in its earlier stages.

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THE ETIOLOGY OF OROYA FEVER AND VERRUGA PERUVIANA *

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MODERN methods of investigation have gradually dispelled much of the obscurity which formerly existed regarding the cause and nature of a number of the more or less imperfectly differentiated fevers which are encountered commonly in tropical and subtropical countries. Through the discovery of the filarial embryo of elephantoid fever in 1863 (by Demarquay), the plasmodium of malaria in 1880 (by Laveran), the *Micrococcus melitensis* of Malta fever in 1886 (by Bruce), the bacillus of bubonic plague in 1894 (by Kitasato and Yersin), the trypanosoma of Gambian fever in 1901 (by Forde and Dutton), the Leishmania of kala-azar or dum-dum fever in 1903 (by Leishman and Donovan), and the spirochæta of African tick fever in 1904 (by Nabarro, Ross and Milne), an accurate diagnosis and differentiation of these febrile conditions became possible. However, the exact nature and cause still remain undetermined of at least a few other febrile diseases which occur more often in tropical countries.

Owing to very recent observations one more malady, namely Oroya fever, probably may now be transferred from this group of fevers of undetermined etiology and placed with the group of febrile afflictions in which the causative organism of the complaint has been recognized. The observations referred to were made during the past summer upon an expedition to South America,[1] the other scientific members of which were Dr. Tyzzer and Mr. Brues of Harvard University, and Dr. Sellards of Johns Hopkins University.

From observations also made during this expedition our

* Delivered February 28, 1914.

understanding of another disease, verruga peruviana, likewise has been greatly increased. The two diseases, Oroya fever and verruga peruviana, were formerly considered to be merely different stages of the same affliction, and hence any discussion of the previous investigations relating to one must necessarily involve more or less the other.

From remote historical times the inhabitants of Peru are said to have suffered severely from an obscure disease characterized by fever, anæmia and a nodular eruption upon the skin. Over four centuries ago, during the reign of the Inca, Huayna Capac, thousands of lives were swept away, supposedly from this malady, and in the history of the conquest of Peru by Zarate, published in 1543, it is stated that the disease is more destructive than smallpox, and almost as disastrous as the plague itself. Later this author mentions that the Portuguese soldiers were afflicted by boils or warts of a very malignant kind, and that not a single person in the army escaped them. De la Vega also relates that during the sixteenth century a quarter of the invading army of Peru under Francisco Pizarro perished from it. In 1842 Archibald Smith[2] called attention again to the disease, and shortly afterward a number of additional papers relating to it were published, among which may be particularly mentioned those of Odriozola[3] in 1858, Dounon[4] in 1871, and Tupper[5] in 1877.

In 1870 a severe outbreak of fever took place among the workmen building the Central Railway between Lima and Oroya, and it is estimated that at least seven thousand individuals died during the epidemic. At this time the complaint received the name of "Oroya fever," although it appears that it was not contracted in Oroya itself. In 1906, out of a force of two thousand men employed in tunnel work for the Central Railway in Peru, two hundred are known to have died of the disease. It appeared to be only necessary for the workmen to spend a single night in the infected districts in order to contract it. Previous to 1885, as has been intimated, there ensued some discussion as to whether Oroya fever and verruga were related to one another, or whether the latter was a distinct disease.

August 27, 1885, Daniel Carrion, a medical student in Lima, and a native of Cerro de Pasco, Peru (a town situated in the mountains far above the localities in which the disease abounds), attempted to solve this problem by vaccinating both his arms with blood from a verruga nodule. It is related that twenty-three days later, he began to suffer from Oroya fever, from which he died sixteen days later, or on October 5th. From this experiment the conclusion was drawn by Peruvian physicians that verruga and Oroya fever were only different stages of the same disease, and this is the opinion which has been held by practically all of them up to the present time, unless our first publication on this subject made several months ago has somewhat modified it. In honor of Carrion's attempt to throw light on the question, the febrile condition which has been regarded as the first stage of the malady is now generally known in Peru as "Carrion's fever." Although it has been stated that Carrion during his illness kept notes, and gave a minute description of his symptoms to his companions, unfortunately it appears that none of these were preserved and published. No accurate record of Carrion's case and of the necropsy is available. It has been asserted since that he died of typhoid fever or of a more acute form of septicæmia, and that perhaps the case from which he infected himself was suffering with Oroya fever as well as verruga at the time.

If we turn our attention to the studies which have been carried on previously in relation to the etiology of these conditions, we find very conflicting opinions, although the majority of writers on this subject have ascribed a bacterial origin to the disease. Izquierdo[6] in 1884 reported the presence of a bacillus in the study of sections of tissues sent him from Peru, and Florez[7] in 1887 from the blood of individuals affected with verruga cultivated on agar-agar a coccus. Odriozola[8] also reported the presence of a bacillus in the blood.

In 1901 and 1902 Barton[9] performed extensive bacteriological investigations, and concluded that in the blood and organs at necropsy of persons dying from Carrion's fever a bacillus was present which, though similar to *Bacillus coli communis*, could

be distinguished from it. This organism, which he regarded as the cause of the disease, was said to give rise either to a fatal septicæmia in animals, or to a verruga-like eruption of the skin. Biffi and Carbajal[10] and Tamayo and Gastiaburú[11] investigated this bacillus, and reported that it was present constantly in persons suffering from Carrion's fever, and was agglutinated by their serum, but was absent from persons suffering from verruga peruviana without fever, whose serum also showed no agglutination of this bacillus. They identified the organisms isolated as belonging in the paratyphoid group. They were unable to confirm Barton's results as to the production in animals of a skin eruption by the bacillus, and concluded that it may be a secondary invader in verruga, or may give rise to the symptoms of a form of fever resembling typhoid which constituted the "fiebre grave" of Carrion.[12] In 1903 Biffi[13] and Gastiaburú[14] noted in stained preparations of the blood the presence of granules in the red blood cells which stained readily with the basic aniline dyes. The form and structure of these bodies was that of a coccobacillus or of irregular granules. In 1905, before the Sociedad Medica Unión Fernandina, Barton[15] described in the red blood cells of two persons sick with severe malignant fever elements similar in morphology to bacilli. In 1909 he noted the presence of these elements in stained blood-specimens in fourteen additional cases and expressed the belief that they were protozoa and probably the specific agent of the infection. In 1909 Gastiaburú and Rebagliati[16] observed the bodies described by Barton and stated that they were probably protozoa and might be regarded as the pathogenic organism in Carrion's disease. Later, De Vecchi,[17] Mayer,[18] Gastiaburú and Rebagliati,[19] Monge,[20] and several other observers have been inclined to believe that the bodies described by Barton were products of cell degeneration. Bassett-Smith[21] and Galli Valerio[22] studied several stained specimens of the blood sent them from Peru. Bassett-Smith concludes that before any opinion is arrived at as to the nature of the inclusions in the red cells, a detailed study of a large number of fresh specimens should be made to see if their presence is

constant in all acute cases. Galli Valerio mentions that in the red cells he observed the bodies previously described, some of which in one specimen resembled *Anaplasma marginale* of cattle. He regards the etiology of verruga peruviana as still undetermined. On the other hand, Nicolle,[23] Letulle,[24] Escomel,[25] and Galli Valerio,[26] found acid-fast bacilli in the lesions of the skin from patients with verruga. Darling,[27] whose studies relating to this disease are based on a necropsy made in Panama the day following the death of a patient with verruga complicated with tuberculosis, suggests that the acid-fast bacilli previously described were really tubercle bacilli occurring in cases complicated by tuberculosis. In the same paper Darling, apparently from an examination of stained blood films not from his own case, but sent him from Peru by Barton, concludes that the slender rodlike bodies in the red cells which he refers to as " x bodies," seemed to represent some unique type of micro-organism. In his own case of verruga no "x bodies" were observed in the preparations, with the possible exception of the smears made from the rib-marrow which he states contained a few rodlike bodies, not definitely in the erythrocytes. Still more recently Gastiaburú and Rebagliati [19] have found in the liver and skin lesions of a verruga patient in the eruptive phase certain bodies, at times endoglobular in leucocytes and other cells, and at other times free, which from their staining reactions and morphologic appearance they regarded as probably organisms of the genus Leishmania. Rebagliati also found certain endoglobular bodies which he considered to be remnants of nuclear disorganization of the erythrocytes, which suggested that Barton's bodies might be chromatin filaments, segregated from the nuclei of erythroblasts. Finally, during the past year, Mayer, da Rocha-Lima and H. Werner,[28] in the study of a case of verruga in Hamburg, believed that they had found bodies resembling Chlamydozoa in the cells in the skin lesions.

Thus it will be seen from this review regarding the previous investigations pursued, that at the time our studies in relation to this subject were undertaken, the etiology of verruga peru-

viana and of Oroya fever was undetermined, and the question was in a confused condition, owing to the fact that the work of previous investigators had not been confirmed.

According to the generally accepted opinion among the physicians of Peru at the time of our arrival in Lima, the disease verruga peruviana in the severe form begins with an initial stage known as the " fiebre grave " of Carrion, which is characterized by a fever which lasts from fifteen to thirty days, profound anæmia, prostration and a high mortality. If the patient does not die in this stage the fever begins to abate, and the eruptive or verruga stage commences. If the eruption is generalized and abundant, then it is stated that the patient is sure to recover. In the chronic or mild form of the disease, which is said to comprise the great proportion of the cases, there is moderate fever of intermittent or remittent character, and pains in the joints are common; more or less anæmia is present. The eruption is said to be the culminating feature in both forms, and it appears under various types which, according to the special characteristics they reveal, are termed " miliary," " nodular," or " mulaire."

After studying these conditions in Peru, we concluded that verruga peruviana and Oroya fever represent two distinct diseases. The former is due to a virus which may be transmitted to animals by direct inoculation and which produces definite lesions in them, whereas the latter is due to an organism parasitic in the red blood corpuscles and endothelial cells sufficiently distinct from the other hæmatozoa to be placed in a new genus. So far this organism has not been successfully transmitted to the lower animals. The parasite which we consider to be the cause of Oroya fever produces in man fever and in severe infections a rapid and very pernicious form of anæmia, which results in extreme prostration and frequently in death. In one of the cases studied by us, which resulted fatally, the red blood cells numbered less than one million per cubic millimetre. At the necropsy in this case, in addition to the evidences of a very severe anæmia, the spleen was enlarged and showed hemorrhagic infarctions. No other organism to which death could be

ascribed was found present. In this uncomplicated case there was no eruption of verrugas. Both intravenous and intratesticular inoculation of rabbits, as well as intravenous inoculation of a monkey, with large amounts of defibrinated blood from severe Oroya fever cases did not produce any noticeable results; and the parasites observed in the blood in the cases in man were not found in the blood of the inoculated animals. The parasites were observed in the blood in man both in fresh and in stained preparations. According to Barton the bodies observed by him could not be detected in the fresh blood, and it has been stated that their refractive index must coincide with that of the erythrocyte. The organisms we observed in the blood consisted of rodlike bodies and more rarely of rounded ones, situated within the red cells.

Fresh Blood Preparations.—Although in fresh blood preparations the organisms are frequently difficult to detect, and at times appear to lie deep in the substance of the red blood cell, nevertheless with good illumination and an oil-immersion apochromatic objective, they may at times be distinctly observed. In form they are rounded or rod-shaped, though the rods are not always straight in outline. The rods measure approximately from 1.5 to 2.5 microns in length and the rounded bodies from 0.5 to 1 micron in diameter. In severe infections, red corpuscles in almost every field of the microscope are invaded by the parasites, and numbers of both rounded bodies and bacillary forms are frequently observed in a single cell. The organisms are endowed with definite motility, which amounts to slow transition and is totally distinct from that of pedesis. To observe this it appears advisable previously to warm the slide and to examine the preparation immediately after it is made. In red cells in which several of the parasites are visible it is easy to observe their frequent change of position within the cell and with reference to one another. The rod-shaped forms were observed to glide slowly in the direction of their long diameter and to exhibit a slight bending in their transition in the red cell, and at times both these and the rounded bodies might be

seen to occupy in turn all portions of it. They were never observed to appear as cross forms, as distinct spirals or markedly S-shaped. Occasionally at the two poles of the organism a dot or beadlike appearance was observed. On account of their small size the rounded bodies are more difficult to describe in fresh specimens and it can only be stated that they change their position within the cell.

Stained and Fixed Preparations.—The rod-shaped forms measure approximately from 1 to 2 microns in length and from 0.2 to 0.5 micron in thickness. They are frequently curved, and occur singly or end to end in pairs, or in chains of three, four and five. When numerous they often lie parallel to one another. V forms, probably representing dividing organisms, are frequent. Y forms are also not uncommon. Cross forms are rare and may be due to organisms being superimposed. The ends of the rods in stained preparations are often more intensely colored. Single rods sometimes show a deep red or purplish granule which may be of the nature of chromatin and which gives the appearance of a swelling at one extremity, the rest of the rod having a more bluish tint. Other rods may be blue throughout or have a deeply stained granule at each end. In organisms occurring in chains the deeply staining granules sometimes give a beaded appearance to the chain. It is evident that the organisms do not all lie in the same plane within the corpuscle, and that certainly many of them are not superimposed, as is believed by Rowley-Lawson to be the case with the malarial parasite in certain stages. This is quite evident from some of the photomicrographs.

The rounded forms measure roughly from 0.3 to 1 micron in diameter. While many of these are rounded, others are slightly oval, or suggest pear-shapes. They occur singly, in pairs, or in groups which suggest previous division.

The red cell may contain a variable number of parasites. In severe infections from one to ten is not unusual as may be seen from the photomicrographs. Nucleated red cells at various stages of development are not uncommon and are sometimes

infected with the parasite. From the anæmia which occurs in this disease and the number of red cells infected with parasites, it seems evident that the red cells containing the parasites are ultimately destroyed.

From the description given above it is evident that we have to do with a species of organism possessing some of the characteristics described for Anaplasma or of Theileria, but also differing widely from other characteristics described for each of these genera. The rounded bodies resemble the Anaplasmata as first illustrated by Theobald Smith [29] in 1891 and later described by Theiler [30] in their form and size, and in the fact that they apparently consist entirely or almost entirely of chromatinic substance. The bacilliform or rod-shaped bodies predominate in our specimens, and in their morphological appearance some of them resemble considerably the rod-shaped bodies observed in certain species of Theileria. However in their staining reactions they are different, the chromatinic substance is not differentiated from the cytoplasmic substance with the same ease, and distinct cross forms have not been observed. Moreover, their movements, so far as could be observed, are entirely unlike those of the piroplasmata. While the organism at first sight might be regarded from its morphology alone as a species of bacterium, this idea is not supported by further study. It is essentially a parasite of the red blood and endothelial cells; attempts to cultivate it on various culture media have been unsuccessful and the inoculation of large amounts of blood from severe cases of Oroya fever has failed to infect a monkey or rabbits.

From the preliminary evidence it appeared that the organism observed in the blood in Oroya fever belonged to a group of micro-organisms intermediate between the protozoa and the bacteria, just as perhaps the Spirochætæ form another such group. It resembles in some of its characteristics the features given for two species of Grahamella N. G. Protista described and classified by Brumpt[31] in October, 1911, although in preparations stained by Giemsa's or Wright's stains the presence of reddish-stained granules and of bluish cytoplasm in many forms

would favor its relationship to the protozoa. We regarded it preferable, however, until further information as to its nature was acquired, to follow Brumpt in his classification of Grahamella, and to go no further than merely to place this species with the Protista. From the descriptions in the literature it appeared that the bodies described by Graham-Smith,[32] Prowazek,[33] and others, in the blood of moles and other animals, and classified by Brumpt [31] and Prowazek [33] as parasites, possibly constituted organisms of species closely related to the one which we encountered in the blood in Oroya fever. As in the genus Grahamella, the organism of Oroya fever is characteristically rod-shaped, and evidently multiplies in one stage by binary transverse division. Apparently, however, the resulting elements do not always separate at once, but remain connected in chains of three, four and five. On the basis of this difference and of other additional characteristics in the life cycle, motility, staining reactions and size, it appeared preferable to propose provisionally for the organism found and studied by us the name of *Bartonella bacilliformis.* The generic name of *Bartonella* was suggested owing to the fact that Barton in 1909 inclined to the belief that the inclusions earlier observed in the red cells were protozoa.

The genus was defined provisionally from the forms studied in the red blood cells as follows: organisms rounded or rod-shaped, sometimes occurring in chains of several dividing forms; reproduction by binary transverse division, and probably by sporulation; cytoplasm and chromatinic substance differentiated with difficulty; endowed with independent motility; living as parasites in the red blood corpuscles and endothelial cells.

Subsequently upon examining sections of the organs from severe fatal cases of Oroya fever evidences of the life cycle were found in the swollen endothelial cells of the lymphatic glands and spleen and from this study it is evident the organism is a species of Protozoa. A detailed description of the life cycle in the endothelial cells will be given in another paper.

While there is not time this evening to refer in detail to the lesions observed in the histological study of sections of the

organs of individuals succumbing from Oroya fever, since these lesions hitherto have not been carefully described, the more important ones will be referred to briefly. Marked changes are encountered in the liver, spleen, bone-marrow, and lymph-glands. The liver shows areas of toxic degeneration which have apparently resulted from the activities of the parasite of the red corpuscles, *Bartonella bacilliformis*, and this fact would suggest that during the course of the disease in addition to the destruction of the red cells by the parasites developing in them another pathological process results from the presence of a toxin in the circulating blood. The action of such a toxin seems to be revealed in the liver by the presence of extensive areas of necrosis of the central type, beginning about the hepatic veins. In these areas many of the liver-cells in sections hardened in Zenker's solution show numerous vacuoles in their cytoplasm which suggest spaces formerly occupied by fat, and in tissues hardened in formalin frozen when cut and stained with Scharlach roth the characteristic stain for fat is obtained in these areas. Distinct zones of these cells containing fat droplets surround central areas of more marked necrosis in which many of the liver-cells are very granular. Other liver-cells about these areas appear homogeneous and hyaline and stain intensely with eosin. Much granular material is present in the sinusoids and numerous endothelial phagocytes frequently containing erythrocytes, erythroblasts and polymorphonuclear leucocytes are present in the periphery of the necrotic areas between the liver-cells. While there is not extensive pigmentation of the liver, moderate amounts of pigment in granules or small masses may be seen within the endothelial phagocytes and in a few endothelial cells lining the sinusoids. Occasionally small granules of pigment may be seen in the liver-cells. Most of this pigment does not give the iron reaction; it is yellowish or brownish in color, and not black. Crystals of hæmatoidin are also present.

The spleen is enlarged and shows numerous infarctions. In sections the edges of the infarcted areas are much congested and show leucocytes migrating into the necrotic tissue. Many of

the veins show thrombosis suggesting endothelial injury from some toxic or infectious agent. At the periphery of many of the splenic nodules just outside the capsule, areas of necrosis are visible in which there is marked deposition of fibrin. There is a large amount of pigment in the spleen, which occurs in small or larger masses and also in fine granules. It is yellowish or yellowish brown in color, and is present in both the endothelial leucocytes and also free in masses between the splenic cells. The pigment is not black, and does not resemble black malarial pigment. It resembles melanin in that it does not give the iron reaction. Whether in Oroya fever the anæmia partially results from the destruction of the red blood cells through the circulation of hæmatin, as W. H. Brown[34] believes to be the case in malaria, at the present time is not clear. *Bartonella bacilliformis* evidently produces no pigmentation in the red cells in the peripheral circulation. No malarial parasites are visible in the splenic sections. The spleen is rich in pulp and much congested; many nucleated red blood corpuscles are visible. Endothelial phagocytes frequently containing red blood cells are also present in abundance. In the bone-marrow there is also striking evidence of phagocytosis by endothelial leucocytes of the red corpuscles in various stages of development, and of polymorphonuclear leucocytes. It seems probable that this extensive phagocytosis which is evident in the liver, spleen and bone-marrow is also an indication of the action of a toxic substance which has led to chemical changes or injury to the engulfed cells, though obviously other theories regarding the cause of this phenomenon may be advanced.

In the lymph-glands the most striking histological feature which at once attracts the attention is the presence of large, swollen, endothelial cells sometimes free in the lumen of the vessel, at other times still attached to its wall. In some instances a single endothelial cell is so swollen that it will almost occlude the entire lumen of the vessel. These cells are in various stages of degeneration. Many of them contain rounded and rod-shaped bodies, and it is apparent that one stage of the parasite is passed within these cells. The details of these changes, how-

ever, will be described by Dr. Tyzzer and myself at length on another occasion.

VERRUGA PERUVIANA

Verruga peruviana is a disease particularly characterized by an eruption, which develops on the skin and occasionally on the mucous membranes, especially of the mouth and throat. At the onset there are malaise, lassitude, depression, pains in the joints, and fever. These symptoms are followed by the cutaneous lesions, which vary greatly in appearance. The distribution of the cutaneous eruption resembles somewhat that seen in yaws; but in many other respects the lesions of the disease are entirely distinct. In uncomplicated cases, neither the parasites of Oroya fever nor those of malaria are present in the blood; though as verruga peruviana is contracted in regions in which Oroya fever and malaria are common diseases among the inhabitants, and where visitors are likely to contract such maladies, it is not surprising that concomitant infections with these parasites frequently occur. Indeed, according to the statistics of Peruvian physicians, in a large percentage of verruga peruviana cases the blood examination shows an infection with malarial parasites; and in one of the twenty cases of verruga peruviana which we studied a concomitant infection with Oroya fever was observed.

The various cutaneous lesions of the disease can be described very briefly. The eruption consists at first of erythematous areas in which groups of small papules soon form. Often small vesicles appear which may be grayish or reddish, or dark blue, according to their degree of vascularity and the character of the blood within them. The areas over which this so-called miliary rash appears are frequently œdematous, and this is most commonly observed on the legs. Unless the disease is arrested the papules grow until they usually measure from 1 to about 5 mm. in diameter. They are usually reddened, raised, and sharply circumscribed from the surrounding tissue, usually discrete, sometimes confluent, and during the most active process of the disease, are of a bright or dark cherry color. The skin over them is tense, translucent and adherent.

Later they assume a gray color or become the color of the skin. As the disease advances, in addition to the cutaneous papules deeper subcutaneous nodules appear, which at first may be quite free from the skin and lie deep in the subcutaneous tissue. Later many of these grow towards the surface and the skin over them becomes involved and adherent. These nodules vary from several millimetres to 3 or 4 centimetres in diameter. The nodular type is especially common about the flexures of the knees and elbows and over the thighs and legs. After these nodules have become adherent to the skin, the skin over them sometimes becomes broken and they may ulcerate, reaching the surface as red fungating masses. These lesions are known as verrugas of the " mulaire " type and often measure several centimetres in diameter. They also may be pedunculated. While the eruption occurs most commonly on the legs and arms and shoulders, it not infrequently appears on the face, forehead and ears. Sometimes it appears on the trunk. The miliary eruption also may occur in the mucous membrane of the mouth. After lasting for several weeks and sometimes for three or four months or even longer, during which time the eruption and nodules may disappear and reappear, that is, true relapses occur, the eruption fades, dries up, and the nodules become absorbed without leaving a scar. In some cases only the miliary eruption appears and convalescence supervenes after several weeks.

The malady has been stated to be confined to the western slopes of the Andes in Peru, though it probably occurs occasionally in southern Ecuador and northern Chili. In the case mentioned by Havilland Hall[35] the disease was contracted in Zaruma, Ecuador. It also seems very probable it is related to, if not identical with, the disease " Angiofibroma cutis circumscriptum contagiosum" described by Bassewitz[36] as occurring in southern Brazil. In Peru the disease is usually confined to the deep-cleft narrow valleys of the Andes lying between two thousand to nine thousand feet above the level of the sea.

The unusual climatic conditions which prevail in these localities—situated as they are so near to the equator and

isolated by mountain ranges—have undoubtedly exerted an influence in producing in some respects a very unusual flora and fauna in these regions; therefore it is not surprising, perhaps, that unusual diseases should be encountered in these localities. The days are very hot and sunny but the sun sets behind the mountains at three or four o'clock in the afternoon and the nights are usually cold. There is frequently a diurnal variation of from 25 to 30 degrees Fahrenheit. Cold mountain streams run through these valleys and the vegetation is abundant only in the vicinity of their banks, owing to the fact that very little rain falls in these regions. The mountain-sides themselves are formed of bare rock and are almost barren of vegetable growth. Cacti and large sprays of heliotrope and a few begonias are commonly found in the crevices beside the rocks.

It is quite evident that verruga peruviana represents an entirely distinct disease, and that it is not a form of frambœsia or of syphilis. In our studies both of these diseases were excluded. The Wassermann reaction was negative with one exception in the cases of verruga which we examined, and no fixation of complement could be obtained when a specific antigen was substituted for the antigen used in the routine Wassermann reaction.

From a study of the pathology of the disease it may be clearly seen that verruga peruviana also should in no way be confused with Oroya fever, or indeed with any other malady, as the lesions are almost pathognomonic. While the lesions encountered in Oroya fever hitherto have not been accurately described, those in verruga have been very extensively studied by various authors and reports upon the histological examination of the cutaneous lesions have been made by Dounon (Cornil et Renaut),[4] Izquierdo,[6] Letulle,[24] Escomel,[25] Tamayo,[11] Hercelles,[28] Biffi,[13] Bindo de Vecchi,[37] and very recently by da Rocha-Lima[39] and by Cole.[40]

Obviously there is not opportunity this evening to review or to compare the observations of these various authors, and I shall confine myself to a brief description of the characteristic

structure of the skin lesions as I have observed them. The very early nodules consist of newly formed blood-vessels lying in œdematous connective tissue. These areas are often very poor in cells, though at different stages of the lesion a variable number of lymphocytes, larger plasma cells, and polymorphonuclear leucocytes are present. The newly formed blood-vessels are often very numerous, and a striking feature in regard to many of them is the small calibre of the vessels in comparison to the number and large amount of protoplasm present in their endothelial cells. In some instances the endothelial cells of the vessels form more than one layer, those on the outside continuing to proliferate. Sometimes the appearance suggests that a capillary vessel has become occluded, and swelling and proliferation of the endothelial cells have resulted. As the lesion progresses there is a very extensive proliferation of these angioblasts which give rise to large islands of closely placed cells. In these areas and about their periphery small numbers of true fibroblasts may be made out. However, the angioblast is the prevailing type of cell in the early verruga nodule. Other writers with the exception of da Rocha-Lima and Cole refer to the prevailing type of cell in the verruga nodule as the fibroblast. Dounon and Izquierdo emphasize the fact that the nodules consist of a structure resembling sarcomatous tissue. Escomel assigns a specific character to these cells and designates them as verruga cells. Bindo de Vecchi insists that they are merely fibroblasts, while Cole describes the prevailing type as the plasma-cell. A careful study of the very early lesions, however, seems to convince one that these cells are true angioblasts. This seems even more clear when one studies the staining reactions of these cells and compares these reactions with those of the endothelial cells of the small blood-vessels, and also studies the progress of development of the lesions. In no other condition does one find such a striking and so characteristic a proliferation of the endothelial cells lining the blood-vessels, as is encountered in the early verruga nodule, and it is this feature which particularly distinguishes the lesion from other pathological processes. Mitotic figures are numer-

ous among these angioblasts. In the older lesions the fibroblasts have gradually invaded the islands of angioblasts and deposited collagen fibres between them. In this way the nodules come more closely to resemble a fibrosarcomatous structure. The verruga nodule, therefore, constitutes a special form of granuloma characterized in the early stages by the formation of new blood-vessels in œdematous connective tissue, and by marked proliferation of angioblastic cells forming islands of closely placed cells, by the invasion of the connective tissue by lymphocytes, plasma-cells, and leucocytes, and as the lesion progresses, by the formation of fibroblasts and the deposit of collagen fibrils.

The disease owes its origin to a virus which may be transmitted to several species of the lower animals. Upon intratesticular inoculation into rabbits, or sometimes into dogs, a characteristic local lesion is produced after an incubation period varying from ten to twenty-two days. By repeated reinoculations of this character, however, the virus becomes gradually attenuated, and finally inoculations of this nature fail. The monkey is by far the most satisfactory animal for inoculation purposes. If the skin over the eye is scarified and a small portion of a verruga nodule rubbed into the abrasions, after an incubation period of usually from ten to twenty days, small papules appear which gradually enlarge and later assume the typical picture of the verruga nodules as seen in human beings. The nodules thus produced also have a similar histological structure to those observed in man. We have transmitted this virus from animal to animal through twelve successive series of monkeys since our departure from South America. In twenty-five monkeys typical lesions have been produced in this manner. Jadassohn and Seiffert [41] in one case of verruga peruviana, and Mayer, da Rocha-Lima and H. Werner[28] in another, also succeeded in transmitting the virus to monkeys through several generations. Cole[40] showed from a study of Jadassohn's case and the material from the animal lesions that the histological structure of the nodules produced in the monkeys was also similar to that of the human lesions.

Numerous cultivation experiments with the virus on artificial media of various kinds have been undertaken. These have usually resulted in failure. Various modifications of Noguchi's and Murphy's methods have been employed. In several instances, however, cultures made in ascitic fluid containing rabbit's testicle to which exudates produced by injections of aleuronat containing leucocytes have been added, together with small fragments of verruga nodules, the cultures have shown certain differences from the control ones, notably in a cloudiness of the media, and in the formation of a fine granular precipitate along the sides and bottom of the tube. This precipitate perhaps resulted from disintegration of the tissues in the culture by the growth of the virus. Apparently it did not constitute itself a living organism, as these cultures revealed no bacteria or other visible micro-organism. In the case of one monkey, No. 22, inoculated with such a culture which had been incubated for sixteen days at 37° C., a small group of reddish papules appeared in a row on each brow after an incubation period of twenty-one days. These papules, however, never developed into as extensive lesions as almost invariably happens when the fresh virus is used and they disappeared in a shorter time. Thus it appears that while the virus was still alive in the culture media, it had evidently become more or less attenuated. Further experiments in cultivation are in progress. When portions of the verruga nodules are finely ground, suspended in water or saline solution, and the resulting mixture passed through Berkefeld filters, the filtrates so far have failed to produce lesions in monkeys, and monkeys inoculated with such filtrates are not rendered immune to the infection. Either the virus has been present in too small amount in the filtrates to give rise to the production of lesions in an animal so relatively insusceptible to the infection as is the monkey, or else it has failed to pass through the pores of the filter. Nevertheless, we have been unable to detect by microscopic examination either in numerous fresh preparations, often examined with the dark field illumination, or in stained preparations made from the lesions or in cultures made from them,

or in sections of the tissues, any definite visible micro-organism.

When the monkey is inoculated with the virus either directly from man or from another monkey, no generalized eruption occurs but only a localized lesion develops. As in the case when these animals are inoculated with smallpox virus a modified form of the disease results. During the past few months Dr. Tyzzer and myself have been able to show that monkeys may be very successfully immunized by a single cutaneous inoculation of the virus, and when such monkeys are reinoculated, no lesion develops. In every instance the monkey vaccinated in this manner has been protected against a second attempt at infection. It therefore seems probable that, by using the virus after several passages through animals, man may also be successfully vaccinated against the disease. This method of vaccination seems even more hopeful, since in one human case that was inoculated with the virus, only localized lesions also developed. It is hoped that this method of vaccination in man in the near future may be given a careful trial in the regions in Peru where the disease is so prevalent.

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THE SIGNIFICANCE OF THE THYMUS GLAND IN GRAVES'S DISEASE*

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IT was perhaps in 1849 that the first experimental proof was brought of the action which a ductless gland might exercise upon the organism. Berthold, professor in Göttingen, transplanted the testicles of young cocks and noted that the birds so treated developed the masculine voice, sexual desire, comb and love of combat. He thus in considerable measure anticipated Brown-Séquard, to whom the doctrine of internal secretions is generally accredited and who, twenty years after Berthold, committed himself to the view that a gland, whether possessed of ducts or not, elaborated substances which were essential to the growth and maintenance of the body and for the preservation of health. It was a memorable meeting of the Société de Biologie of Paris at which, two decades after his first pronouncement, this super-gifted man related in support of his views the results of experiments made upon himself. He testified, as you recall, that following the injection of testicular juice he observed an astonishing revivification of his physical and mental powers.

One of the least understood and most complicated of the various distinct but intimately associated mechanisms at work in sustaining the orderly activity of the animal body is what has been termed the chemical correlation. Each organ, each tissue and each cell of the organism may exert a chemical influence upon some other far-removed tissue of the body and thus aid in bringing about the adaptations and readjustments essential for the integrity and life of the whole.

The acid chyme passing into the duodenum stimulates the

* Delivered March 14, 1914.

epithelial cells of the mucosa to the production of a substance which by way of the blood-stream calls forth responses in pancreas, intestines and liver, exciting the secretion of pancreatic and intestinal juices and of bile. Bayliss and Starling proposed the name " hormones " (*ὁρμάω*, to awaken, stir up) for such physiological products as serve to arouse the various organs to activity.

The structures which produce hormones are commonly called endocrine (*κρινειν*, to separate) organs or glands of internal secretion.

Meltzer believes that, strictly speaking, such products of the chemical action of organs as must be regarded as terminal resultants of the decomposition processes, as the waste or by-products of metamorphosis, products which enter the circulation merely for the purpose of prompt elimination, should be differentiated from those substances which, formed in specific manner in individual or special organs, are taken up by the circulation and carried to distant parts, there to fulfil particular functions. Only these latter substances should, in the opinion of Meltzer, Gley and others, properly be designated as hormones, and Gley has proposed the appellation " par-hormone " for the decomposition products which invariably result from cellular activity and which as irritants might exercise a deleterious influence did not the fluids and tissues of the body form definite protective reactions.

Hormones can in two ways exercise their functions; either by direct action upon the tissues or by means of the nervous system. In addition to these relatively direct methods of manifesting their actions upon the terminal apparatus it is quite certain that the secretion of a given gland may act indirectly or through the agency of other hormonopoetic organs. For example, the influence of the thyroid by way of the thymus upon the activities of the stomach. To what extent the visceral nervous system plays a part and what may be the sequence of events in the course of the complicated interaction of the endocrine glands may in a measure be determined by experimental studies in metabolism. Thus it has been ascertained that

in animals the glycosuria which develops on administration of adrenalin vanishes after excision of the thyroids. If these animals are now fed with thyroid extract the glycosuria reappears. On the other hand, in the pancreatectomized animals excision of the thyroid does not cause the glycosuria to disappear.

Studies in metabolism have taught us that the thyroid and pancreas on the one hand and the pancreas and chromaffine system on the other reciprocally inhibit. Between the thyroid and chromaffine system, however, there exists a reciprocal potentiation. Hence when hyperthyroidism gives rise to glycosuria it may conceivably do so by inhibiting the pancreas. In a still more roundabout way the thymus via thyroid, via pancreas might possibly bring about glycosuria unless, perhaps, the influence of the thymus upon the adrenals were protective.

Modern pharmacology regards as antagonists the sympathetic and autonomic * nervous systems. As the sympathetic system possesses in adrenalin a specific pharmacological stimulant so analogously the autonomic system has in pilocarpine and muscarine its specific irritants. One can by the administration of thyroid extract produce symptoms in animals and man which strikingly resemble the stimulating effects of pilocarpine or muscarine (sweating, diarrhœa, disturbances in respiration, lymphocytosis, eosinophilia, etc.). The standpoint has accordingly been taken that in the thyroid there is a constituent which produces an effect similar to that of the poisons of the pilocarpine group. In favor of this view speaks, especially, the well-known antagonism between thyroid extracts and atropine, which is an antidote for pilocarpine and muscarine. It has now been attempted on the basis of these physiological facts, which indicate that from the thyroid gland impulses may be sent out along the tracts of the sympathetic as well as the autonomic system, to distinguish the symptoms of Graves's disease which might be due to irritation of the sympathetic

* This word is used in the restricted sense, and not as intended by Langley, who proposed the term *autonomic* for the entire sympathetic system.

from those which might be attributed to autonomic stimulation. (Eppinger.)

SYMPATHETICO-TONIC BASEDOW SYMPTOMS

1. Pronounced protrusio bulbi.
2. Von Graefe, absent.
3. Löwi's phenomenon, positive.
4. Möbius, positive.
5. Dry bulbs.
6. Greatly increased activity of the heart with less pronounced subjective disturbances.
7. Sweating and diarrhœa, absent.
8. Falling out of hair.
9. Eosinophilia, absent.
10. Inclination to fever.
11. Alimentary glycosuria.
12. Refractory behavior to pilocarpine.

VAGO-TONIC SYMPTOMS

1. Relatively moderate degree of tachycardia.
2. Pronounced subjective heart symptoms.
3. Von Graefe, definite.
4. Wide lid-clefts.
5. Möbius, absent.
6. Slight protrusio bulbi.
7. Increased lachrymation.
8. Profuse sweating.
9. Diarrhœa.
10. Disturbances of digestion.
11. Eosinophilia, likely.
12. Alimentary glycosuria, absent.
13. No adrenalin-glycosuria.
14. Pigmentation.

There are certain exceptional, more or less sharply differentiated cases of Graves's disease which every physician and surgeon who has actively interested himself in the subject must vividly recall. During the past twenty-three years in a series of about five hundred I have seen perhaps twenty which were

especially typical of their kind. The characteristic features which most of them presented were great emaciation, dilatation of the heart, sweating, diarrhœa, relatively slight exophthalmos, not excessive tachycardia, small goitre, and frequently a peculiar greyish bronze-hued skin.

Confronted with a case of this kind, I have repeatedly said to my assistants: "Here is another of these puzzling, dreadful cases. The patient is not highly thyrotoxic; the thyroid is hard, not large, nor pronouncedly vascular; if a lobectomy is performed she may die, but probably not with the stormy symptoms which suggest extreme thyroid intoxication. If death occurs it may be sudden, possibly not until several days after the operation, and perhaps when the patient seems to be convalescing."

In one of these cases death occurred four days after the ligation of a single artery under local anæsthesia. The operation, including the injection of the local anæsthetic and the sewing up of the tiny wound, required exactly ten minutes. One night, as I have said, four days after the operation, and a few minutes after the nurse had charted the pulse as 90, the patient awoke with a start, sat upright in bed, gasped for breath and died. The following case died suddenly about thirty hours after a thyroid lobectomy when we had ceased to be apprehensive about her.

Case I.—A. V. C. (No. 33,010). Æt. 47. Admitted October 8, 1913. Except for whooping-cough at the age of 15, mumps at 19 and measles at 21, patient states that she has enjoyed good health until about four years ago.

In January, 1909, patient experienced on swallowing the sensation of a "lump in her throat." At about the same time she was seized with a severe headache, became very nervous, suffered from palpitation and vomited several times. Thinks she had fever in this attack. Almost immediately the thyroid gland "began to swell." She was quite ill for three or four months but in May of the same year felt as well as ever. Her recovery was so complete that she considered herself in normal health until January, 1913, when she was taken ill again quite suddenly with precisely the same symptoms as in the previous attack. Palpitation and shortness of breath were more pronounced than at first; diarrhœa, vomiting and difficulty in swallowing also became troublesome symptoms. Cessation of the menses occurred about that time. Since July 1, 1913, she has been confined to bed.

Examination.—Patient is greatly emaciated (weight 67 lbs.); is very nervous, constantly jerking about and tossing her head; there is fine tremor of the fingers and tongue. She has decided mental aberration, ideas of persecution and grandeur; has bad dreams; talks to angels; is confident that her mind is affected, etc. Has staring expression; is very nervous; the whole body seems to be shaken by the heart-beat. The skin is ashy-bronze in hue. There is slight exophthalmos; definite von Graefe; convergence fair; rather wide palpebral clefts, but sclera covered. Joffroy sign absent; pupils react to light and accommodation. The hair is dry, grey, thin and falling out. Thyroid uniformly and moderately enlarged and very firm. Faint bruit but no thrill over thyroid arteries. No palpable enlargement of any lymphatic glands. The carotids throb forcibly. Veins of neck pulsate. There is great emaciation; deep supra- and subclavicular fossæ. Movements of respiration symmetrical. Lungs clear. Respiration 28. Thymus: there is no definite retro-manubrial dulness; X-ray negative. The heart is greatly dilated. There is a soft blowing systolic murmur over the whole cardiac area; it is heard best at apex. Pulse 140 to 155 and over per minute, and irregular. Arteries not hardened, blood-pressure 115 to 125. Abdomen scaphoid. Marked aortic pulsation. Edge of liver not palpable.

Blood.—White corpuscles, 13,360; red, 4,404,000; hæmoglobin, 61 per cent.

October 13, 1913. *Differential Blood Count.* 250 cells counted.

	Cells	Percentage	Actual number of each in cmm.
Polymorphonuclear neutrophiles	128	51.2	6,840
Small mononuclears	86	34.4	4,595
Large mononuclears	21	8.4	1,222
Transitionals	5	2.0	267
Eosinophiles	3	1.2	160
Basophiles	1	.4	64
Unclassified	7	2.8	374
Myelocytes	2	.8	106

October 20, 1913. Patient vomited frequently until five days ago. Has two to three or more watery stools a day. Has persistent cough.

October 21, 1913. *Operation I. Ligation of Left Inferior Thyroid Artery.* The artery was easily found and tied. It was as large as a vertebral artery. Patient's pulse rose to 200 and over during the operation under novocaine, nitrous oxide gas, and a few drops of ether.

October 24, 1913. Patient remarkably improved since the operation. Restlessness greatly relieved. Vomiting has ceased. Pulse now averages only 100.

October 25, 1913. *Operation II. Gas. Ligation of Right Inferior Thyroid Artery.* Artery very large, very thin-walled and blue like a vein. Operation required only a few minutes.

October 30, 1913. Patient has improved rapidly since second operation. Is quite cheerful. Hair regaining lustre.

October 30, 1913. *Differential Blood Count.* 250 cells counted. White blood cells, 8,300.

	Cells	Percentage	Actual number of each in cmm.
Polymorphonuclear neutrophiles..	122	48.8	3,984
Large mononuclears	37	14.8	121
Small mononuclears	69	27.6	2,201
Transitionals	21	8.4	704
Eosinophiles	1	.4	41

November 4, 1913. Marked improvement in patient's general condition. Pulse 88. Patient eats heartily; neither vomiting nor nausea.

November 11, 1913. No pronounced change in patient's condition during the past week. Appetite large but there has been no gain in weight (60 lbs.).

November 16, 1913. Patient has gained 4¾ lbs. in past five days.

November 24, 1913. No definite improvement during past week.

November 25, 1913. *Operation III. Right Lobectomy.* Gas and a few drops of ether. Operation simple and rapid. Patient's pulse rose only occasionally to 180, but was usually about 160 during the operation. At the first operation it rose to 200 and over. At the end of the operation the patient's condition was excellent.

November 26, 1913. *Differential Blood Count.* 250 cells counted. White blood cells, 18,400.

	Cells	Percentage	Actual number of each in cmm.
Polymorphonuclears	155	62.0	11,408.0
Small mononuclears	51	20.4	3,753.6
Large mononuclears	22	8.8	1,619.2
Transitionals	8	3.2	588.8
Eosinophiles	5	2.0	368.0
Basophiles	5	2.0	368.0
Unidentified	4	1.6	294.4

November 26, 1913. Patient had a fairly comfortable night and seemed to be convalescing satisfactorily until 1.20 P.M. to-day when she was found sitting up cyanotic and gasping for breath, coughing, trying to clear her throat, excited and apprehensive. Pulse very irregular. Respirations 40 to 50. Area of precordial dulness markedly increased, extending 4 cm. to the right and 10 cm. to the left of the median line. At 1.50 P.M. she became quiet, seemed fairly comfortable and dozed. Pulse 140 to 180 and irregular in force and rhythm. Cyanosis less.

3.40 P.M. Suddenly seized with another dyspnœic attack. Respirations became more labored and at 4.05 P.M. ceased. The heart apparently

stopped beating about one minute before the cessation of respiration. The temperature rose to 101.5° F.

Autopsy (Dr. Reid).—Subject strikingly emaciated. Left lobe of thyroid slightly enlarged; small slice of right lobe in region of the parathyroids. Cardiac dilatation. Emphysema of both lungs; bronchopneumonia. Large thymus extending down to the auricles. Fatty degeneration and atrophy of liver; small adenoma of liver. Mesenteric glands rather larger than normal; otherwise no enlargement elsewhere of the lymphatic glands. Dilated, atrophic stomach. The thymus gland was thick but not broad enough to cast an X-ray shadow beyond the manubrium. It was triangular in shape, the finely tapered apex reaching to the top of the manubrium, the broad base extending to and partly covering the auricles of the heart.

The endocrine glands have been carefully studied microscopically and will probably be reported upon at other times by the various men to whom these organs were specially entrusted. I was particularly interested to find that in the thyroid glands the follicles instead of showing the usual picture found in exophthalmic goitre were for the most part circular and distended with densely staining colloid without vacuolation. The epithelial cells were low-cuboidal or flat. In places, however, throughout the gland the colloid was rarefied and the cells high.

In this patient the vago-tonic symptoms predominated (diarrhœa, vomiting, small goitre, slight exophthalmos, definite von Graefe, Möbius not pronounced, intense subjective symptoms, pigmentation).

Was this a case of thymus death?

Physiologists, pharmacologists, pathologists and clinicians are rapidly reflecting, consciously and unconsciously, helpful light for the interpretation of the clinical phenomena and dire results in cases belonging more or less definitely to this category.

In 1910 Meltzer * made the following cautious statement concerning the thymus gland: "With our present knowledge of the importance of the other ductless glands we are hardly justified in assuming that the thymus is a worthless fetal

* Meltzer: "Animal Experimentation in Relation to Our Knowledge of Secretions, Especially Internal Secretions," Proc. of the Path. Soc. of Phila., Sept., 1910.

remnant. But we have to acknowledge that as yet there are no reliable observations or experiments which indicate clearly that the thymus has a function in post-uterine life."

Von Mikulicz (1895) called attention to the occurrence of enlarged thymus in severe cases of exophthalmic goitre, and Rehn (1899) suggested that it might be well to attack the thymus gland surgically in this disease. In 1908 Hart expressed the opinion that abnormal activity of the thymus gland might produce the clinical picture of Graves's disease.

It was my good fortune to be present when Garrè, at the Fortieth Congress of the Deutsche Gesellschaft für Chirurgie (1911), took part in the discussion of Kocher's paper on exophthalmic goitre in order to report the first instance in which the thymus had been primarily removed for the cure of this disease. Although well aware of the fact that a " persistent " or revivified thymus had repeatedly been observed in " Basedow " he was astonished to learn from the statistics obtained by his assistant, Dr. Capelle, that a " thymus persistens hyperplastica " had been found in 95 per cent. of the fatal cases, whether death was due simply to the severity of the disease, or occurred during the operation, or within twenty-four hours after the strumectomy.

So impressed was Garrè with these findings that he finally determined to test the effect of thymectomy in cases in which there was good reason to believe that the thymus was enlarged. It seemed to him that a severe and florid example of Basedow should be selected for the experiment and that the thyroid gland should be unmolested in the operation the purpose of which was to determine the effect of a thymectomy upon the symptoms of the disease.

The result in his first case was as follows: Clinically no definite influence on the struma, the exophthalmos or the eye symptoms, but an unequivocal improvement in the general condition as expressed by the " éclatant " quieting of the heart's action, rapid increase in weight, and a complete regression of the Kocher blood-picture, the lymphocytes falling from 40 to 25 and then to 10 per cent.

In his second case Garrè performed simultaneously a hemistrumectomy and thymectomy.

The striking results which followed the first of these operations forced upon him the thought that the hyperplastic thymus in exophthalmic goitre displays an action essentially similar to that of the thyroid and that the thymus persistens aggravates the symptoms of the disease.

Professor Garrè gave to his assistant, Dr. Capelle, credit for the work and the thought which led them both to these conclusions, which he said finds essential support in the proof by Klose that in the thymus-substance there is a heart-poison. Aside from the special action of the thymus there exist certainly, said Garrè, important reciprocal relations between these two glands, and for the following reasons:

1. After thymus extirpation, the blood-picture, which by Kocher is considered characteristic of Graves's disease, returned to normal precisely as after a successful strumectomy.

2. His assistant, Dr. Bayer, had recently demonstrated that intraperitoneal injections of the expressed juices of the thyroid as well as of the thymus produce the Kocher blood-picture, whereas the juices of colloid struma and of normal thymus influence the blood-picture to a much less extent.

3. They found in a thyroid gland which had been removed six months after a thymectomy microscopical evidence of regressive changes.

4. Gebele had announced at the previous Congress that the prompt implantation of normal thymus in thyroidectomized dogs prevented the appearance of cachexia strumipriva.

5. He was able to state, thanks to the permission of Dr. Bircher, that the latter had twice produced the typical Basedow picture by the intraperitoneal implantation of the fresh, pathologically hyperplastic thymus. These and other facts made him unable to subscribe to the generally accepted view of Möbius that the thyroid is alone responsible for the disease. On the other hand he was not prepared to take the extreme view of Hart that there was a purely thymogenic form of Basedow.

I have presented in such detail the views so briefly and cautiously expressed in 1911 by the highly gifted director of the surgical clinic in Bonn because this contribution of Garrè and Capelle marks an epoch in the developing story of Graves's disease and its treatment the importance of which is as yet not realized.

During the past three years the research work on the thymus, which already had been considerable, has assumed great proportions, and for surgeons interested in Graves's disease this gland has become a theme on which their attention may well be focused.

And now, just a few weeks ago, appears a most convincing paper by von Haberer, the youngest of Billroth's assistants, and, until his promotion to the directorship of the Innsbruck surgical clinic, first assistant to von Eiselsberg in Vienna, who is also a distinguished product of the school of Billroth. The results of thymectomy in von Haberer's case, number 3, are so remarkable as to be almost unbelievable were they recounted by an authority less eminent and trustworthy.

Case 3 (von Haberer *).—Merchant, æt. 30; in his earlier years athletic; of late overworked in his business, but well until the autumn of 1909, when, after an attack of fever, he noticed a marked increase in the frequency of his pulse, loss of zeal for work and a feeling of general bodily discomfort. About six months later he observed that his eyes were abnormally prominent, that he was becoming nervous and tremulous and subject to attacks of profuse sweating, and that he experienced feelings of cold in the legs. The greatly increased and irregular action of his heart caused him especial uneasiness. In the spring of 1911 a part of the thyroid gland was removed, and thereafter, for a time, his symptoms, the tachycardia excepted, were somewhat relieved. In the winter of 1911, after unusual business stress, the heart symptoms became greatly intensified and hæmoptysis and dyspnœa supervened. The dyspnœa became so great that the patient was apprehensive at times lest he choke to death. He sought relief at the hands of Professor Kocher in Berne, who ligated the thyroid arteries of the unremoved lobe. No relief was obtained from the arterial ligation; on the contrary the patient's condition became alarmingly worse. He frequently had attacks in which a bloody froth

* Mitteilungen aus den Grenzgebieten der Med. u. Chir., Bd. xxvii. Heft 2, p. 210.

was expectorated and consciousness lost. He finally could not walk without provoking these attacks.

On the seventh of December, 1912, the patient presented himself at the clinic in Innsbruck of von Haberer. He was cyanotic, gasping for breath and covered with cold sweat; the pulse could not be felt in the peripheral arteries. Fearing that the man might die in his office, Professor von Haberer had him hastily despatched to a sanatorium. After a short rest in bed in a half-sitting posture (the only one endurable for the patient) the pulse returned at the wrist. It was 160, very irregular and unequal. Exophthalmos was particularly pronounced on the right side; skin and mucous membranes cyanosed; slight von Graefe and Möbius signs on both sides; tremor and great dyspnœa; right lobe of thyroid very slightly enlarged; left lobe not palpable. Over both lungs signs of œdema and congestion-bronchitis; heart much enlarged, extending on the left side four finger-breadths outside of the mammary line. Liver greatly increased in size, its lower thick border extending as low as the navel. Slight but definite icterus. Compression of the trachea by the thymus was excluded both by percussion and the Röntgen ray. There was, however, behind the manubrium a triangular shadow, which, without demarcation, blended with the shadow of the heart. Percussion revealed no unusual dulness.

The patient begged piteously for operation, indifferent to its dangers; desirous only to be relieved of his great distress. Professor von Haberer refused to interfere surgically, being convinced that if an operation were undertaken at that time it would result fatally. The physician called in consultation agreed that the patient's condition was hopeless, the heart being exhausted. The thyroid symptoms he regarded as a complication of relatively minor importance and believed that medication would accomplish nothing for the heart. The patient was kept in bed and treated with diuretin and digotoxin. In the course of ten days the condition of the lungs was somewhat improved, but the pulse, never dropping below 140, remained irregular with frequent periods of galloping rhythm. The differential blood count gave polynuclears, 57 per cent.; small lymphocytes, 27.6 per cent.; large lymphocytes, mononuclears and transitionals, 12.3 per cent.; eosinophiles, 2.9 per cent.; mast cells, 0.2 per cent.; coagulation was slightly delayed.

December 19, 1912. All were still agreed, and the patient assured, that he could not survive an operation of any sort. Nevertheless, von Haberer, having in mind the somewhat analogous case of Garrè's, finally decided to yield to the patient's entreaties, and on December 19th, under local anæsthesia, succeeded after prolonged search in extracting from behind the manubrium a piece of tissue 3 cm. long and ½ cm. thick, which resembled fat and macroscopically seemed to contain no thymus gland. Greatly disappointed at the result of the operation and believing

that the last hope for the patient had vanished, von Haberer closed the wound. Towards evening of the same day the patient announced that he felt much better and that his dyspnœa had disappeared. On the fourth day after the operation he became much worse; the pulse was feebler and more frequent, the liver increased in size and the icterus deepened. The following morning, however, and each day thereafter improvement was noted, and on the third of January, 1913, two weeks after the operation, the patient was discharged from the hospital. Three months later (April, 1913) he returned for examination. The surgeons and physicians were astonished at the result. The apex beat was in the mammary line, the pulse 84, although still irregular; the cyanosis and icterus had disappeared; the liver was of normal size, and the color of the skin was good and fresh. The subjective condition was as satisfactory as the objective. The patient declared that he felt perfectly well; he could work as tirelessly as ever and could even climb mountains. In September of the same year this patient wrote that he had climbed for sport a mountain over 7,500 feet high.

Three primary thymectomies have been reported, all of them from Garrè's clinic. To these may, however, be added Sauerbruch's case in which only the ligation of one superior thyroid artery preceded the operation on the thymus. In all of these cases the effect of the operation upon the general condition of the patient and upon the blood-picture was unequivocally beneficial. Of the combined operation, thyroidectomy plus thymectomy, eight cases have been reported by von Haberer; and Capelle and Bayer state that several times in the clinic of Garrè this operation has been performed.

Klose states in his recent book on the thymus gland that he has in five cases of Basedow's disease resected the thymus. In each instance there was striking improvement in the patient's general condition and return of the blood-picture to the normal. I have excised a small portion of the thymus with one lobe of the thyroid in two cases of which I shall speak later.

It has been our practice of late in the strumectomy operations to investigate the contents of the space between the trachea and the manubrium with the object of determining the possible presence of an enlarged thymus, and in a few instances we have made a fairly thorough search for this gland. Only twice have we found it enlarged; in both instances we should have been

disappointed had there been no traces of thymus and our faith in some of the most important strands of the complicated web which the pharmacologists, the physiologists and the clinicians are weaving to represent the interdependent activities of the endocrine glands would have been shaken.

Case II.—H. P. (Sur. No. 31,648.) Æt. 13. Admitted to the hospital March 6, 1913.

Complains of difficulty in breathing, "feeling lazy," and inability to work. During the past four or five years he has had attacks of vertigo, occasionally with loss of consciousness; also attacks of nausea and vomiting preceded usually by headache. Patient might remain in bed for a week after such attacks.

Patient believes that his present illness began two years ago. He noticed first a swelling in the neck which has gradually increased in size; then began to have shortness of breath and the sensation of pressure on the trachea. He could not read aloud for any length of time. He could not run or ascend stairs quickly. During the past five months he has had dyspnœa on lying down and has been unable to sleep on less than three pillows. Ambition for work and play is lost. He is unable to talk loud and finds it impossible to shout. Has a hoarse cough and desire to clear the throat. For the past four or five months he has experienced some difficulty in the swallowing of solid foods, and at times even of liquids; his bowels are evacuated four or five times a day. He has occasional attacks of vomiting before breakfast. Has become irritable. Has not observed palpitation of the heart. Does not perspire excessively.

Physical Examination.—Boy is well nourished and well developed. The hair is oily; the skin neither abnormally dry nor abnormally moist. There is no exophthalmos; the von Graefe sign is positive at times; the other eye-signs are absent. The thyroid gland is enlarged to perhaps three or four times its normal size. There is definite retro-manubrial dulness. The X-ray shows a suggestive shadow. A soft systolic bruit is heard over a very slightly enlarged heart. The pulse is regular, 80 beats per minute. Tremor absent. Cervical, inguinal, submaxillary and axillary glands are palpable.

March 7, 1913. *Differential Blood Count.* 300 cells counted.

	Cells	Percentage
Polymorphonuclear neutrophiles	160	53.3
Small mononuclears	102	34.0
Large mononuclears	23	7.6
Transitionals	8	2.7
Eosinophiles	3	1.0
Basophiles	2	0.7
Unclassified	2	0.7

March 18, 1913. *Operation. Excision of Right Lobe and Isthmus of Thyroid and a Small Piece of Thymus.*

March 26, 1913. *Differential Blood Count.*

	Cells	Percentage
Polymorphonuclear neutrophiles	183	61.0
Small mononuclears	86	28.6
Large mononuclears	15	5.0
Transitionals	7	2.33
Eosinophiles	2	.6+
Basophiles	2	.6+
Unclassified	5	1.6

December 11, 1913 (nine months after operation). *Differential Blood Count.*

Polymorphonuclears	68.0 per cent.
Small mononuclears	23.0 per cent.
Large mononuclears	5.0 per cent.
Transitionals	3.0 per cent.
Eosinophiles	1.0 per cent.

February, 1914: *Examination.* The boy's health is almost restored. He is able to do a full day's work but is rather more fatigued by it than are other boys of the same age. He can sleep on one pillow and has had no attacks of vertigo, nausea or vomiting since the operation. The cough and difficulty in swallowing have disappeared. The diarrhœa has ceased.

As the operation in this case was a combined one, the great improvement which followed it cannot definitely be attributed to resection of the thymus gland alone. The portion of thymus resected was, however, so small that I am inclined to believe that the strumectomy rather than the thymectomy was chiefly responsible for the good result.

Case III.—G. B. (No. 33,918). Æt. 12 years. Admitted to the hospital February 12, 1914.

Has had most of the infectious diseases of childhood (whooping-cough, measles, scarlet fever, chicken-pox, mumps), all before she was seven years old. Occasionally has sore throat but has never had a definite attack of tonsillitis. Function of eyes and ears normal. Has always been a nervous child with poor appetite. Digestion apparently good, although she has each year two or three "bilious attacks" with nausea and vomiting. For the past five years the mother has had difficulty in fitting the collars of her child's dresses, but not until a few months ago did she notice that the neck was enlarged. The eyes have always been prominent, protruding at times more than at others. For the past three or four years the child has complained of a feeling of fulness in the neck, especially when tired and nervous.

Examination.—A rather pale, sallow-looking, frail girl. Expression alert and intelligent. She is unusually clever in conversation. Not obviously nervous at present. Hair normally lustrous. Hands and feet warm and rather moist. No pigmentation of skin. Dermatographia. No dilatation of superficial vessels of forehead or temples. Possibly slight fulness of veins of upper eyelids. Eyes decidedly prominent. Sclera well covered by lids. Palpebral clefts 1 cm. A suggestive stare. Von Graefe doubtful. No other eye-signs. Pupils react to light and accommodation. Slight tremor of tongue, none of fingers. Pulse regular; 100 beats per minute. Accentuated throbbing of the carotids, particularly of the right carotid. Dilatation of superficial veins of neck on both sides. Neck enlarged, rather more so on the right than on the left side. Both lobes of thyroid palpable, the right more definitely than the left. The isthmus is prominent and measures 2.5 cm. vertically. There is neither bruit nor thrill in the gland. Circumference of neck over the isthmus is 28 cm. The entire gland seems of normal or rather soft consistence. There is a suspicion of retro-manubrial dulness. The X-ray reveals, however, no shadow suggestive of the thymus gland.

Tonsils markedly enlarged; crypts visible. Posterior cervical and axillary glands are palpable. Finger tips are slightly clubbed. Child complains of palpitation of the heart and shortness of breath on exertion. States that on two or three occasions she has been obliged at night to sit upright in bed to relieve a feeling of slight suffocation. Has usually two and sometimes three stools a day. Has never been constipated.

February 12, 1914. *Differential Blood Count.* White blood cells, 7,000. Hæmoglobin, 92 per cent.

	Cells	Percentage
Polymorphonuclear neutrophiles	120	60.0
Small mononuclears	71	35.5
Large mononuclears	5	2.5
Transitionals	3	1.5
Eosinophiles	1	.5

February 19, 1914. *Operation. Resected Right Lobe of Thyroid and a Small Piece of Thymus Gland.* The right lobe of the thyroid, about two and one-half times the normal size, was resected according to our regular method, a portion of the posterior part being left to protect the parathyroids and the recurrent laryngeal nerve.

The thymus gland was found to extend upwards almost to the inferior pole of the right lobe of the thyroid. The piece of the thymus resected was hardly larger than the child's little finger.

February 21, 1914 (two days after operation). *Differential Blood Count.*

	Cells	Percentage
Polymorphonuclear neutrophiles	154	77.0
Small mononuclears	25	12.5
Large mononuclears	11	5.5
Transitionals	10	5.0

It is interesting to note the fall in mononucleosis. Nine days before the operation the percentage of mononuclears was 38; two days after the operation it had fallen to 18. The child and mother are greatly pleased with the general improvement which they think has taken place since the operation. It is too soon for us to judge of this.

It is the belief of the few who have expressed an opinion on the subject that the over-activity of the thymus manifests itself chiefly or only after the hyperthyroidism has existed for some time—that it is not observed in the early stage of the disease.

In my patients No. II and III the thymus symptoms predominated (attacks of dyspnœa, diarrhœa, no tachycardia, not much enlargement of the thyroid and not very definite eye symptoms), so one cannot but feel that the hyperthymusism may have been primary in these cases and that later on the thyroid symptoms might have predominated. We shall try to follow carefully the subsequent history of these children; and as only a part of one lobe of the thyroid and a very small portion of the thymus of each child was removed we may not have influenced profoundly the natural development of the disease.

A case upon which a double thyroid lobectomy was done two years ago returned recently for examination. The thymus being still enlarged it was treated for four hours by the emanations of 1,300 milligrammes of radium applied over sixteen squares. Although there has been after ten days no reduction in the size or density of the X-ray shadow, two thyroid nodules, as large as filberts, hypertrophied remnants of isthmus, entirely disappeared within twenty-four hours. Dr. Burnam, who applied the radium at Dr. Kelly's Sanatorium, believes that the thyroid nodules vanished because of the inhibited activity of the thymus. He thinks it is exceedingly unlikely that the emanations could have directly affected the thyroid.

The results of the combined operations have been, without exception, remarkably good; unmistakably better, I should say, than we ordinarily obtain from the operation upon the thyroid gland alone. Particularly striking has been the relative absence of the reaction which is usually observed in the thirty-six or forty-eight hours following thyroid lobectomy. The postoperative course in the experience of von Haberer could not be distinguished from that observed after operations for ordinary struma. This seems the more remarkable because in all these cases there was the complication of an enlarged and persistent thymus. The improvement was immediate and so strikingly pronounced that I agree with von Haberer in believing that it must be attributed to something more than mere accident.

That the thymus plays an important part in Graves's disease has, I think, been demonstrated beyond question by the results which have followed thymectomy. That some sort of relation exists between the two organs we have further evidence from the physical examination of the non-fatal cases, from the autopsy table, and from experiments on animals.

Palpation just above the manubrium, particularly pressure downwards towards the mediastinum, and over-extension of the head, may be complained of by patients with persistent thymus on account of the shortness of breath occasioned by these manœuvres. The Röntgen-ray and the percussion-note over the area occupied by the thymus may give useful information; but the absence of both dulness and shadow does not exclude the presence of a persistent gland, nor do we know as yet how small a thymus may be responsible for symptoms.

It has been estimated as a result of non-operative clinical examination that in about 40 per cent. of all cases of exophthalmic goitre the thymus is persistent. The actual percentage it remains possibly for the surgeon to determine by systematic exploration. We have, I think, no absolute evidence that the thymus may be completely wanting in a case of Graves's disease; and since, as I have stated, we do not know how small a fragment of this gland may suffice to play a part in the disease, we are not as yet in a position to assert that exophthalmic

goitre may exist entirely uninfluenced by the thymus. Von Haberer, however, reports an outspoken case in which careful operative search failed to reveal its presence.

Enlarged thymus not infrequently accompanies colloid goitre. Attention was called by Astley Cooper and by Virchow to this association. The question naturally suggests itself, has the persistent thymus the same significance in both these forms of goitre, one of which we hold accountable for symptoms of hyperthyroidism, whereas with the other the picture of underactivity of the thyroid is associated? Is it not conceivable that the persistent thymus in colloid goitre may in some cases represent responses from time to time to periods of perhaps overlooked hyper-function of the thyroid, and if so, that indeed for the "Kropfherz," a contributing factor may be found in the thymus?

From the postmortem examination of cases of exophthalmic goitre which have died of intercurrent disease it has been ascertained that the thymus gland is persistent in about 82 per cent. of them; and in those cases which have died of heart failure after operation enlargement has been found, as already stated, in about 95 per cent. Probably it will be ascertained that the percentage has been under-estimated for the reason that enlargement of the thymus may not hitherto have been so completely noted or looked for as it will be in the future. Then too we must bear in mind the important lesson taught us from von Haberer's case No. 3, that the severest thymotoxic symptoms may be caused by fragments of thymus so small as to be unrecognizable as such by the naked eye.

At the Johns Hopkins Hospital there has been only one opportunity to make an autopsy on a case of Graves's disease which died after operation. In this instance, as I have mentioned, a long, thick thymus gland was found tapering out from near the top of the manubrium sterni to the auricles of the heart.

What are the particular symptoms of Graves's disease which indicate a preponderate influence of the thymus? Eppinger, Garrè, von Haberer, Klose, Capelle, Bayer, van Noorden, Jr.,

and indeed almost every clinician who has familiarized himself with the literature would say it is the vago-tonic symptoms. But do we know quite definitely what the vago-tonic symptoms are? And in case they should be determined, shall we be in a position to assert that the thymus is or is not alone responsible for them?

Naturally it will fall to the lot of the surgeon to discover which of the symptoms are dissipated by the removal of the thymus; but even when this has been ascertained the proof is not furnished that no other organ could have had a part directly or indirectly in their causation.

Let us consider, for example, the protrusion of the eyeball, a symptom which according to MacCallum and others may be caused by stimulation of the sympathetic. If it is true that this is a sympathico-tonic symptom, and if the thymus were activated solely by the autonomic system, the excision of this gland might not be expected to affect directly and promptly the exophthalmos. But as a matter of fact recession of the eyeball in at least one of von Haberer's cases was much more prompt than has perhaps ever been observed after strumectomy alone. The effect on the thyroid gland, particularly as to its vascularity, seems to have been quite as striking in von Haberer's experience; it must, however, be remembered that except in one case the combined operation was performed.

To discuss the grounds on which the various signs have been assigned to their special groups would carry us beyond the purpose of this paper. I might say, however, by way of illustration, a word with reference to the incomplete convergence of the eyes. This, the Möbius sign, is assigned to the sympathico-tonic group. It was explained by the late Dr. Landström and his supporters as due to the contraction of the Müller-Landström muscle of the orbit which is supposed to be responsible for the protrusion of the eyeball and by the particular arrangement of its fibres to embarrass the action of the internal rectus. There are some, and especially certain countrymen of Landström, who do not consider the demonstration complete that the Müller-Landström muscle is responsible for the exophthalmos. Furthermore, as I have said, the eyeball may promptly

recede after thymectomy, and with its recession the Möbius sign may vanish, which is contrary to what might have been expected of a sympathico-tonic symptom after the excision of an organ activated chiefly by the autonomic or vago-sympathetic system.

RESULTS OF OPERATIONS FOR BASEDOW'S DISEASE *

Year	Authors	Number of cases	Per cent. of cures	Per cent. considerable improvement	Per cent. slight improvement	Per cent. no improvement	Per cent. of deaths
1896	Schulz	20	90.0	....	....	5.0	5.0
1898	Wolf	9	....	66.5	....	....	22.5
	Helferich	6	66.6	16.7	....	....	....
1900	Reinbach (v.Mikulicz)	18	66.5	22.5	....	5.5	5.5
1902	Witmer (Krönlein)	23	40.9	36.2	9.2	9.2	9.2
	Th. Kocher	59	76.0	14.0	....	3.3	6.7
1903	Curtis	11	60.0	10.0	....	....	30.0
1904	Mayo	40	67.5	17.5	....	....	15.0
1905	Lessing (König)	8	50.2	....	37.3	....	12.5
	Hartley	21	87.5	....	....	....	12.5
1906	K. Schulze (Riedel)	50	72.0	12.0	....	2.0	14.0
	A. Kocher	167	93.7	....	....	....	6.3
1907	Itzina (Hildebrand)	7	85.7	....	....	14.3	....
	Mayo (only new cases)	136	78.2	19.6	....	....	2.2
	Landström	54	50.2	15.3	....	29.0	5.5
1908	Moses (Garrè)	28	16.9	41.6	24.9	12.5	4.1
	Klemm	32	93.2	....	3.4	3.4	....
	Th. Kocher	153	....	98.7	....	....	1.3
	MacCosh	22	14.5	72.7	8.2	4.6	4.6
1909	Hänel	21	38.1	42.8	....	....	....
1911	Sudeck	26	84.6	4.0	....	....	4.0
	Baruch	40	72.5	12.5	....	15.0	15.8
	v. Eiselsberg	44	61.4	34.1	....	4.0	....
	Enderlen	40	70.0	20.0	....	2.2	2.2
1912	Klose	61	75.5	9.8	....	1.6	13.1
	Weispfennig	30	60.0	6.6	....	23.3	10.0

* Klose: Die Basedowsche Krankheit. Ergeb. d. Inn. Med. u. Kinderheilk., Band X, 1913.

Surgeons the world over have learned from an experience which is now very large that the majority of patients afflicted with exophthalmic goitre may be relieved of their symptoms by strumectomy, but that in a certain percentage of the cases the cure may be incomplete even after resection of the greater part of both lobes of the thyroid gland. (Halsted.)

I have performed perhaps 650 operations upon about 500 patients with Graves's disease. A one-sided lobectomy, the operation ordinarily done, has resulted in an approximate cure in possibly 60 per cent. of my cases, but in at least 25 per cent. the patient has not been sufficiently relieved by a one-sided lobectomy to resume her full duties, and in certainly more than 60 per cent. (possibly 70 or 80 per cent.) there remain symptoms of over-activity of the thyroid or thymus or of both of these glands sufficiently pronounced to be detected by the expert clinician.

The results of my experience as regards the cure of Graves's disease by one-sided lobectomy are quite at variance with the views of other surgeons.

In some 47 cases in which the improvement was altogether unsatisfactory after the excision of one lobe the other was removed. Of particular interest to me has been the observation, which we have made several times, that definite improvement may not be observed until both lobes have been almost completely excised. In a number of cases—all of them severe—in which the preliminary ligation of three or four arteries plus a single lobectomy was followed by little or perhaps inappreciable benefit the symptoms vanished, almost magically, on the removal of the remaining lobe. I should explain, parenthetically, that I never excise the entire lobe; a small slice is always left posteriorly to protect the parathyroid glands, for the reason that the chances are considerable that the other side may have to be operated upon. It is undoubtedly because surgeons have so universally confined their operations to the excision of one lobe plus, perhaps, the ligation of an artery of the other, that the results have not been better than they are. And you will agree with me that it is rather absurd to conclude that if the excision of an arbitrary amount of a gland supposed to be chiefly responsible for the symptoms did not cure or relieve them it would be useless to remove more of the offending organ.

Now although all of the symptoms including the exophthalmos may be cured by strumectomy, the blood-picture may remain unchanged, at least for a considerable period. We have,

for example, five cases in our wards at present whose mononucleosis is as great and in one of them greater than before operation. In one the four arteries have been tied and one lobe resected. In two both lobes have been excised, and in the fourth, a very serious case, the inferior arteries were first ligated, then the superior arteries; next the right lobe and then the left was resected. This patient's health has already been almost completely restored but the mononucleosis is 53 per cent. (small mononuclears, 34 per cent., large mononuclears, 19 per cent.), 4 per cent. higher than on admission.*

Baruch, Sudeck, Klose, Melchoir, Lampé and Liesegang and others would seem to have brought proof from extensive clinical observations that the pathological blood changes in Basedow's disease remain uninfluenced by the excision of the thyroid gland.

Klose states it is universally conceded that all the symptoms of Graves's disease may disappear after thyroidectomy but that the disordered blood-picture is supposed to remain unchanged, and hence if we desire a hæmatological cure we must attack, surgically, the thymus. Another particular indication, according to Klose, for the excision or destruction of the thymus is the fact that in Graves's disease this gland is qualitatively and not merely quantitatively altered and may cause toxic as well as mechanical injury to the heart.

Borchardt a little more than a year ago reported † the result of his studies in the medical clinic of Lichtheim at Königsberg of the blood-picture in diseases of the endocrine glands. He examined 31 cases of Graves's disease, ten of status thymico-lymphaticus, five of simple goitre, fifteen of myxœdema (two of his own, and thirteen in the literature), thirteen of disease of the hypophysis (three of his own, ten in the literature), and five of Addison's disease (two of his own, three in the literature).

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* These and many other cases controvert the opinion of von Lier, who regards a lymphocytosis of 40 per cent. as a contraindication to operation.

† Borchardt: "Uber das Blutbild bei Erkrankungen der Drüsen mit Innerer Secretion und seine Beziehungen zum Status thymico-lymphaticus." Deutsches Archiv f. klin. Med., Bd. 106, 1912.

Borchardt found that in all diseases of the gland of internal secretion there was in the great majority of the cases an increase in the mononuclear cells, especially of the lymphocytes. There was leucopænia in about half of the cases, and approximately as often eosinophilia.

Since in all diseases of the thyroid, hypophysis and adrenals signs of status thymico-lymphaticus were established, he concludes that the changes in the blood-picture are to be assigned to a status lymphaticus.

My observations do not permit me to accept *in toto* the views of Klose, Borchardt and the many others who attribute the lymphocytosis to the thymus. Of the forty-seven or more cases above referred to, operated upon during the past twenty years, in which I found it necessary to remove both lobes of the thyroid gland in order to relieve sufficiently the symptoms, about one-half have returned within the past two years for examination. In all of these, with perhaps two or three exceptions, the blood-picture is approximately normal. Of particular interest are the findings in a case at present in the hospital operated upon two years ago (double thyroid lobectomy). This patient was so ill when first admitted to the hospital, that I debated for some days as to the advisability of performing a lobectomy without the preliminary ligation of arteries. Her symptoms were predominantly vago-tonic, but there were no signs of enlargement of the thymus. There was a mononucleosis of 53 per cent.

The improvement which followed the resection of one lobe being unsatisfactory the remaining lobe was excised. The blood-picture was at the time only slightly altered by these operations, although there was marked improvement in her general condition.

Now, after two years, she has returned to the hospital for examination. She is almost restored to health and is able to perform all of her laborious household duties. She complains particularly of a sense of pressure behind the manubrium to which she attributes in a measure her shortness of breath at times. Percussion and the X-ray now indicate definitely an

enlarged thymus. The blood-picture is, however, normal. (Mononucleosis, 30 per cent.)

Another patient (Miss G. C.) has just come from Texas for reëxamination, in response to a telegram from me. A year ago when admitted to Dr. Barker's service in the hospital she was acutely and desperately ill and was promptly transferred to the surgical side. The thyroid arteries were ligated, two at a time, and then, one after the other, the thyroid lobes were resected. Vago-tonic and sympathico-tonic symptoms were about equally pronounced. There was extreme exophthamos with all of the eye-signs. Pulse 140–150; slight enlargement of the heart. Sweating, diarrhœa, nausea and vomiting, intestinal gas, bad dreams. Dyspnœa, vertigo and very severe muscular cramps. Mononucleosis, 51 per cent. There was no evidence of thymus enlargement.

Now, after a year, she has gained greatly in weight, is, in fact, a little too fleshy. Her general health is fairly good and is gradually improving. But her condition is not satisfactory. Her pulse is 100–110, she suffers from dyspnœa on slight exertion, has very little energy and is greatly concerned about the exophthalmos which is still a conspicuous disfigurement.* The blood-picture is, however, normal. (Mononuclears, 28 per cent.)

From the facts gleaned at the autopsy table, from experiments on animals and above all from the results following primary thymectomies we have convincing evidence that the thymus gland may play an important part in Graves's disease, and in some cases assume the title rôle. Some of the most puzzling features of the disease are made possible of interpretation by the discovery of the influence which the thymus may exert.

That the secretions of the two organs concerned in the production of the Basedow picture have a relationship there can be little doubt. The injections and implantations in ani-

* May 26. Within the past two months the patient's thymus has been treated with radium and the X-ray. The improvement has been quite marvellous. The exophthalmos has almost entirely disappeared and the patient considers herself well.

mals made by Svehle, Bircher, Bayer, Basch and Gebele demonstrate conclusively that the thymus and thyroid possess in common certain fundamental physiological properties.

Antagonistic factors are also at work in the two glands, the presence of which is indicated sometimes directly and sometimes indirectly by the behavior of other organs. There are few in this audience who are not familiar with the already famous experiments of Gudernatsch. To tadpoles equally developed, he fed to some the thymus of the calf, to others the thyroid of beeves. As a result of the thymus feeding the tadpoles increased greatly in size without differentiation or change in form. The creatures fed with thyroid promptly put forth arms and legs and otherwise rapidly took on the features of the frog. In the relation of the thymus and thyroid to the genital sphere and to the adrenals we find indications of a possible antagonism of some sort between the two glands. It is a well-attested fact that gravidity exercises a favorable influence upon the symptoms of Graves's disease. Basedow, himself, made this observation. This would seem to indicate that in pregnancy, in which unusual demands are made upon the thyroid, an excessive secretion from this gland can be utilized.

Between the thymus and ovaries there is experimental evidence of a possible functional antagonism. Thus Paton, Soli, Klose, Vogt and others have observed after thymectomy an increase in the weight of the ovaries, and according to Tandler and Gross there is abnormal persistence of the thymus in eunuchs.

Eppinger * relates of a case of Graves's disease of mild form that immediately after castration toxic symptoms of threatening severity set in. He states that in the literature (reference not given) he has found a similar case. Of like significance probably is the fact that during the climacterium a mild may be converted into a severe form of Graves's disease.

Four or five years ago I was consulted in the North Carolina

* Eppinger: "Die Basedowsche Krankheit," Handbuch d. Neurologie, Bd. IV, Specielle Neurologie, III, p. 62.

mountains, by a native woman (Mrs. B.), who stated that for the previous six months she had been able to swallow nothing but liquids and for a week or more nothing but water. She was greatly emaciated and so weak that she could hardly stand. She was confident that the obstruction was just behind the "Adam's apple." The thyroid and cricoid cartilages were strikingly prominent, so much so that I confidently expected to feel a carcinomatous mass back of the larynx. I could feel nothing, however, except possibly an indefinite, soft mass of the presence of which I was not absolutely certain. She refused to come to the hospital, saying that she preferred to die at home. A year later I was surprised to find her robust and in perfect health. The difficulty in swallowing began to diminish soon after her visit to me, and in a month or two she was able to take food of all kinds as well as ever. The relief seemed to come quite promptly after the complete cessation of the menses. I concluded that a retrotracheal or retroœsophageal portion of thyroid had become enlarged during the climacterium.

As further evidence of an antagonism between the secretions of the ovaries and thymus we may mention the excessive lymphocytosis which has been observed by Klose to follow the injection of Basedow-thymus after oöphorectomy. Klose injected 5 ccm. of thymus juice expressed from the gland of a Basedow patient into a spayed bitch whose lymphocytes after castration had risen to 32 per cent. Immediately after the injection there developed symptoms of severe Basedow intoxication. Shortly before death, which occurred one hour after the injection, the lymphocyte percentage was 64.

In one of my dogs (No. 9) which for sixteen months had been deprived of both thyroid lobes and all the parathyroids except a graft, Dr. MacCallum found complete absence of spermatogenesis. The testis as a whole was atrophic and spongy, but the interstitial cells of Leydig, although inconspicuous and perhaps somewhat degenerated, were still present in abundance.

What, then, is the relation of the thymus to the thyroid in Graves's disease? As stated by Capelle, the thymus can hardly

be an " Erfolgsorgan " (a terminal apparatus), which enlarges simply in response to a stimulus from the specific gland. It is not merely antagonistic or compensatory (loose terms) to the thyroid, for if so its excision should be attended with an increase of the Basedow-symptoms. These organs have, however, some sort of reciprocal relation. The effect of excision of the thyroid upon the thymus and of the latter upon the former organ has not been definitely determined.

In the dog (No. 9) just referred to, in which both of the thyroid lobes and all of the parathyroids had been excised, the thymus was carefully examined by Dr. MacCallum, who at that time could have had no knowledge of its possible importance in Graves's disease. His report is as follows:

> There is quite abundant thymus tissue. In the thymus there are some cysts, one of them quite large and lined by ciliated epithelium. Ciliated cavities of small size are found quite frequently through the tissue. I do not see any Hassall's bodies unless these might represent them. The thymus is not in the acme of its development, but has undergone a certain amount of atrophy.

Tatum found that the thymus of rabbits atrophied after excision of the thyroid.

As to the effect upon the thyroid of total excision of the thymus there is also uncertainty. The histological picture presented by the thyroid, seven months after total excision by Dr. McClure of my staff, and Dr. Park, Dr. Howland's assistant, of the thymus in a puppy, æt. 3 months, seems to be identical with that obtained by Matti and Klose, and interpreted by them as hyperplasia. On comparing the sections of the thyroids of Dr. McClure's dogs, of the control with that of the thymectomized animal, I should say that the changes in the latter indicate overactivity. These changes consist chiefly in entire disappearance of the colloid, and great increase in the height of the cells. The follicles are perhaps a little smaller in the hyperactive gland than in the control and involutions are not conspicuous.

One can hardly be too cautious in assigning causes for the

appearances found in one gland after excision of another, or in the remains of a gland after the resection of a part of the same. I am prompted to say this from experiences of my own, having particularly in mind our efforts to find the cause of the almost invariable hypertrophy of the thyroid in our dogs experimented upon twenty-six years ago and to explain its absence after identically the same experiments during the past two years.

The pigmentation which has so emphatically arrested our attention in certain cases of Graves's disease deserves, I think, greater consideration than it has hitherto been accorded.

Our interest in this symptom has vastly increased now that we believe, I may say know, that the thymus may be an important factor in the disease.

As to the frequency of the occurrence of abnormal discoloration of the skin the statements of the various authors do not agree. Sattler, who gives the matter full consideration in his classical work on the symptomatology of Graves's disease, places it at 18 per cent. Kocher finds abnormal pigmentation of the skin once in eight cases; Friedrich Müller observed it in four out of five of the serious cases; Murray noticed a more or less pronounced pigmentation of the skin forty-two times in a series of 180 cases. There are some who think that this symptom rarely occurs. I have observed it chiefly in the instances of severe and of long-standing disease, and on reviewing the histories of my patients am impressed with the fact, as it seems to me, that the pigmentation has been more frequent in the vago-tonic type of the disease.

This observation accords with what might be expected from animal experimentation and from the relation which has been observed by pathologists and clinicians of the thymus to the adrenals in status thymico-lymphaticus and in Addison's disease. Dr. Samuel J. Crowe of my staff finds as a result of careful search of the records of the pathological department of the Johns Hopkins Hospital that in all the cases of status thymico-lymphaticus there is a note to the effect that the adrenals

were atrophied, and that in Addison's disease hypertrophy of the thymus is almost invariably recorded.

Boignet and Calogero and Matzoukis found that excision of the adrenals was followed by hypertrophy of the thymus. Soli, Matti and, I think, Klose noted enlargement of the adrenals after thymectomy. Wastenson states that involution of the thymus may occur in consequence of the injection of the extract of the medulla and cortex of the adrenals. Possibly the extract of the medulla alone might have produced similar results, for Matti states that it is the medullary portion of the adrenals which hypertrophies in dogs deprived of the thymus gland.

Matti found, further, in his own laboratory an indirect confirmation of the above-mentioned experimental data in that two animals with strikingly pronounced thymus-hyperplasia following extirpation of the spleen showed an extraordinary diminution in the amount of adrenal-medulla. In this constant reaction between the thymus and adrenal glands depressor influences are espied.

TREATMENT

Primary thymectomies uncomplicated by strumectomy, and secondary thymectomies in cases not sufficiently relieved by resection of both lobes of the thyroid would be the operations of choice for those searching for the essence of the Basedow thymus. The combined operation would be avoided by them as much as possible until it became more definitely known how profoundly and in what particulars the thymus may influence the disease. The excision of even a very small piece of either gland for microscopical examination, in the course of operation upon the other, might vitiate the experiment. For example, in our Case II (boy æt. 13) the resection of a portion of thymus hardly larger than one's thumb was followed by almost complete relief of the symptoms, including a return of the blood-picture to normal—symptoms which would be considered thymo- rather than thyrotoxic.

We are debating what should be done in a case desperately ill in which there are reasons for believing that the thymus is

enlarged. Should we first ligate the thyroid arteries at one or more operations or perform primarily a thymectomy? * The ligation of one and perhaps even of two arteries is less of a proceeding than the excision or resection of the thymus, but is it not possible that the removal of a part of the gland the more specifically responsible for the disease might be better withstood by the patient than an operation of less magnitude upon the other? In our Case I, for example, the ligations of the inferior thyroid arteries were followed by very little reaction and by great improvement in the general condition of the patient. Would it not have been better, we are asking ourselves, to have performed a thymectomy either primarily in this case, or in preference to the thyroid lobectomy which was done subsequent to the ligations? The patient, you will recall, died suddenly one day after the strumectomy. These are questions to which further experience must give the answer.

I may say that except in the instance reported (Case I) we have had no death from a lobectomy which had been preceded by preliminary ligation of one or more of the thyroid arteries. And even in the case just cited I am inclined to think that death might not have occurred had I ligated the superior as well as the inferior vessels. I believe from my own experience that, disregarding for the moment the question of thymus resection, we have absolute proof of the advisability of ligating the thyroid arteries, as advised by Kocher, in the severest forms of Graves's disease, indeed in all cases where there seems to be the slightest ground for fear that the patient might not withstand lobectomy. Most surgeons have abandoned or not practised preliminary ligation of the arteries, contending that the repeated operations are more troublesome to the surgeon, are unpopular with the patient and yield no better results. As to the last point I am sure they are mistaken; as to the weight to be attached to the other two, each must be his own judge.

* May 26, 1914. For the past two months we have been treating the thymus gland of selected cases of Graves's disease with the X-ray. The results will be reported later.

From the point of view of the research worker the combined operation, thyroidectomy plus thymectomy, should, as I have said, not be done, but when having excised one lobe of the thyroid I have found myself actually confronted at the operating table with an enlarged thymus I have felt compelled for the patient's sake to resect it.

There is much reason for hope that radium and the Röntgen ray may give us the solution of this question. The blood of Basedow patients who have been treated with the X-ray shows remarkably little mononucleosis (Klose). Klose, Arella, Heineke, Peters and others have shown that under the influence of the X-ray the thymus rapidly undergoes involution, an involution which is so extensive that Klose expressly warns against radiation of the thymus region in children. The relative absence of lymphocytosis in Basedow patients whose goitres have been treated with the X-ray is attributed by Klose to the influence of the rays upon the thymus. In a case referred to earlier in the lecture it was mentioned that two nodules, remains of the thyroid isthmus, vanished promptly after prolonged exposure of the thymus to 1,300 mg. of radium. The gradual enlargement of these nodules had caused the patient great anxiety, and she was the first to notice that they had disappeared. Furthermore, she no longer experienced the feeling of oppression behind the sternum which had been a source of constant annoyance and occasionally of distress.

I have touched my subject only very lightly at some of the higher points. Hardly enough has been said even to make it clear that an enormous amount of work underlies the facts which we at present possess. It must be evident to every one, however, that there reigns the greatest confusion on the subject of the function of the glands of internal secretion.

Fortunately the ardor for research on our globe is not diminished by the conviction that we are laboring in the wake of workers infinite in numbers on countless worlds who have carried their investigations millions of years beyond the stage reached by us, and are rapidly progressing towards an ultimate solution which may never be reached.

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